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# URINARY ANTISEPTICS

## WITH A REPORT ON THE EXPERIMENTAL EXAMINATION OF 35 ANILINE DYES.

BY

ALBERT T. SUDMAN.

A THESIS

PRESENTED TO THE FACULTY OF THE

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## THE IDEAL URINARY ANTISEPTIC

In 1918 when starting his investigations on urinary antiseptics, Davis (7) set the following requirements for an ideal urinary antiseptic;

1. It should be chemically stable.
2. It should be non-toxic.
3. It should be non-irritating to the urinary tract.
4. It should exert an antiseptic action in high dilution in urine of any reaction.
5. It should be eliminated in high percentage by the kidney.

Leonard, in 1924, (19) suggested a modification of the fifth requirement and added a sixth;

5. It should be eliminated in the urine in sufficient concentration to exert a local antiseptic action, and at a rate by which continuous antiseptic action may be attained.
6. It should be administrable by mouth.

## THE NEED FOR AN IDEAL URINARY ANTISEPTIC

Since 1918 Davis, Young, White and others (7,8,13,14,31, 32,33) working in the Brady Urological Institute have been striving to find a drug which would meet the above qualifications and which would also clinically produce the results expected of it. From this work resulted the introduction into the field of urinary antisepsis of acriflavine (14) and mercurochrome (33). Leonard (19) in 1924, brought forward

hexylresorcinol as meeting the requirements of an ideal urinary antiseptic. And now more recently pyridium has been reported by Ostromislensky (25) as being of value in this work.

But as late as 1926 Davis (11) states, "the ideal internal urinary antiseptic of proven clinical efficiency does not exist." In England, Brown and Dukes (3) in 1929 stressed the inadequacy of present antiseptics. Thus today there still remains a need for an ideal urinary antiseptic which will meet the clinical and experimental requirements without reservations.

## LIMITATIONS OF URINARY ANTISEPTICS

The discussion of this subject is not undertaken without a conception of the limitations of the effect of any urinary antiseptic. Davis says (7), "It is worthy of repetition that the various sedative and antiseptic drugs discussed are indicated only as temporary or palliative measures, during the more acute stages, or pending investigation." Thus too much emphasis cannot be placed upon the need for careful and thorough physical and urological examinations in all infections of the urinary tract. It would be folly to expect a permanent cure merely by the use of urinary antiseptics in a case of pyelitis or cystitis with a highly active focus of infection continuously flooding the genito-urinary system with virulent organisms. The ideal in any form of treatment in any branches of medicine is to determine and eradicate the etiological factor, this is the responsibility of the physician and not the requisite of drugs.

Practically all authors regard the vast majority of cases of urinary infection as secondary to prevailing infection or pathology. The presence or absence of urinary retention; from hypertrophied prostate, urethral strictures or other causes; of ureteral obstruction, from calculus or stricture; of bladder pathology, calculi, or foreign bodies; of a focus of infection within the genitourinary tract or at some other body site, such as teeth, tonsils or sinuses; or any other pathological lesion must be determined in all infections of the genitourinary tract. Urinary antiseptics only offer aid in the management of these cases while they are being treated according to the method indicated by careful and complete examinations.

## AIM OF THIS THESIS

It is the aim of this thesis: 1. To briefly discuss the most commonly used urinary antiseptics, namely, hexamethylenamine, acriflavine, mercurochrome, hexyloresorcinol and pyridium. 2. To report on the experimental examination of 35 aniline dyes as urinary antiseptics. This work was done in the Department of Clinical Research under the direction of Dr. Edwin Davis, Dr. F. Lowell Dunn and Dr. C. V. Morgan.

## HEXAMETHYLENAMINE

Hexamethylenamine (urotropin) was first used as an urinary antiseptic by Nicolaier (24) in 1895. It was soon conceded that the antiseptic action resulted from the production of formaldehyde as a decomposition product. Variable results were reported from the use of this drug in the next few years. Burnam's (5), in 1912, and Hinman's (17), in 1913 reports on

detailed experimental and clinical investigations of urotropin, practically fixed the status of this drug. The necessity of an acid urine and the need for massive doses in most cases were shown. Hinman showed that the dilution of formaldehyde was low in most cases, only 17 per cent ( of the cases ) produced complete bacteriostasis while 5 per cent were germicidal. He showed the need for restricting fluids and the failure of the drug to be of any value at the kidney level. Sutton (28) in 1923 pointed out that the drug is valueless in patients with retention catheters and in urinary fistulas.

Today we find the conception of the value and limitations of urotropin is quite well recognized. It is usually given in doses of 15 to 25 grains, three or four times daily. This drug is only active in acid urine and the percentage of formaldehyde liberated is usually directly proportional to the acidity. (The concentration of formaldehyde is seldom estimated to be higher than 1 part in 5,000) Thus the necessity of the administration of sodium acid phosphate, benzoic acids or benzoates, ammonium chloride or some other drug to acidify the urine, in most cases is readily seen. It is used orally in practically all instances but intravenous administration has been used, (2). Urotropin has a definite value as a prophylactic agent before instrumentation. It has also given good results in alterating with other drugs preferring an alkaline urine especially in the chronic pyogenic infections of the genitourinary tract.

## ACRIFLAVINE

Acriflavine was first shown to have high antiseptic properties by Browning and others (4) in 1917. But the application of this drug to the field of urinary antiseptics was made by Davis and White (14) in 1918. They showed the high bacteriostatic action of acriflavine in urine of alkaline reaction and the excretion of antiseptic urine by rabbits following intravenous injection. The same year Davis and Harrell (12) reported on the treatment of gonorrhea with acriflavine. Then again in 1921, Davis (8) demonstrated the excretion of antiseptic urine (if reaction was alkaline) by man following the oral administration of acriflavine. In 1924 he (10) showed the clinical limitations of acriflavine in genitourinary infections. He concludes that acriflavine in 0.1 gram doses along with sodium bicarbonate is of distinct value in acute urinary infections but in chronic infections the results are variable and inconstant. Nausea, vomiting and diarrhea sometimes accompany the administration of acriflavine and occasionally reach the stage where they contraindicate the use of the drug.

Acriflavine is now given in a dose of one and a half grains three times a day in keratin coated pills or capsules. (The latter is to prevent the gastric disturbances which develop when acriflavine comes in contact with the stomach.) It is much more active in an alkaline medium consequently sodium bicarbonate is administered at the same time. It is also used locally for irrigation of urethra and bladder in a

dilution of 1 to 4000. This drug is frequently used in alternating with urotropin in the treatment of persistent genitourinary infections.

MERCUROCHROME - 220

The original work with mercurochrome - 220 was published by Young, White and Schwartz (33) in 1919, at which time they pointed out its antiseptic properties. Hill and Colston (16) showed the antiseptic properties of urine from rabbits following intravenous injection. Young, Hill and Scott (32) reported on the oral administration of mercurochrome in 1924. They stated that germicidal effects were produced but felt more investigation was needed before any conclusions could be drawn. Thomas and Wang (29) report that 36.6 per cent of 60 tests inhibited or killed bacterial growth after oral administration of 300 mgms. of mercurochrome three times a day. Moderate to severe gastrointestinal symptoms were also reported in some cases.

Striking results have been reported with the intravenous use of mercurochrome by various writers in genitourinary infections and cases of septicemia. This phase has been quite thoroughly reviewed by Young (31) in 1925 and Braasch and Bumpus (2) in 1926. They used a dosage of 5 mgms. per kilogram of body weight. Rather severe toxic manifestations were reported in some cases. The latter authors state "It is evident that mercurochrome should be used intravenously only if emergency demands it."

Mercurochromes chief use at the present time is as a local antiseptic. It is quite highly recommended for local

application and bladder instillation by various authors. For this work it is used in dilutions from one-half of one per cent to two per cent.

## HEXYLRESORCINOL

In 1924, Leonard (19) reported on hexylresorcinol as a new urinary antiseptic. At this time he demonstrated the variation in antiseptic action with changes in chemical structure of alkyl resorcinols. He found hexylresorcinol to be the most efficient antiseptic experimentally and that clinically results were obtained that were promising. Henline, in 1925, (15) reported 92 per cent cures in a series of cases by use of hexylresorcinol. In a series of articles Leonard and others (20,21 and 22) have brought out the necessity of an adequate dosage, of withholding fluids and of avoiding the use of sodium bicarbonate in treating urinary infections with hexylresorcinol. A variation of opinions exists among various urologists as to the value of hexylresorcinol. Braasch states (1), "Unfortunately it has not proved to be of such value in vivo as had been hoped for." He is supported in this view by Davis (6), Pugh (26) and Walther (30).

Hexylresorcinol is used some at the present time with the same variable results. It is administered in doses of 0.45 to 0.60 gram three times daily. It is occasionally rotated with urotropin in treating chronic urinary infections with favorable results.

## PYRIDIUM

Pyridium, a recent highly advertised but little investigated

drug, will be discussed briefly. It was first described by Ostromislensky in 1926 (25) in his small book "The Scientific Basis of Chemotherapy." He advocated its use in infections of the eye, nose, throat, and gums, in furuncles, in gonorrhea and in other genitourinary infections. Since its introduction it has not been given much experimental scrutiny. Favorable results have been reported by Walther (30) in 1929, stressing the following values. 1. Its marked power of stimulation of epithelial tissues. 2. Its bactericidal action. 3. Its ability to penetrate. 4. Its rapid elimination through the genitourinary tract. He also states "Never in my 18 years of urological practice have I seen any drug act upon bacteriuria so promptly and so effectively as pyridium." Pugh (26), (27) reports favorable results in clinical work with pyridium. However Thomas and Wang (29) state that there seems to be little experimental proof for the use of this drug. In Germany the clinical use of pyridium is given consideration by Koster (18) and Neuberger (23).

It is used in doses from 0.1 to 0.2 gram three times per day. It seems that more careful clinical and experimental examination is needed before the status of pyridium as a urinary antiseptic is known.

## EXPERIMENTAL EXAMINATION OF 35 ANILINE DYES

### REASONS FOR EXAMINATION OF DYES

In 1921, Davis (9) gave the following reasons for the investigation of aniline dyes. (1) A large number of these

compounds are available. (2) They are colored hence they can be readily detected and quantitatively estimated in the urine. (3) Previous work by others has shown that dyes have antiseptic properties with therapeutic possibilities. To these may be added (4) Dyes with their wide variation in chemical structure offer a field for further study of the relationship between antiseptic power and chemical structure. (5) The chemical structures of the various dyes quite easily avail themselves to alteration - thus suggesting the possibility of synthesizing a suitable drug from the most promising dyes. (6) The ideal urinary antiseptic is yet to be found.

With these ideas in mind the examination of 35 aniline dyes by means of the method to be discussed was undertaken.

1. DETERMINATION OF ANTISEPTIC VALUE ON AGAR

Two organisms were used namely, Staphylococcus aureus and Bacillus coli. The agar was prepared according to the directions of Stitt and tubed in 9 cc. amounts. To each tube before plating in sterile petri dishes was added, 1 cc. of a 1 to 1000 aqueous solution of the dye being tested, under sterile precautions and by means of a sterile pipette. After it had cooled it was inoculated with a stroke of 24 hour broth cultures of each Bacillus coli and Staphylococcus aureus. After 24 hours the results were recorded as shown in Table I; + signifies growth, - signifies no growth.

2. DETERMINATION OF ANTISEPTIC PROPERTY IN URINE

The next step was to find out if the antiseptic action was present in urine as well as in agar. It was also desired

to have the dye examined in both acid and alkaline urine. Thus two samples of voided urine were taken and one titrated to pH of 6.4 with tenth normal hydrochloric acid and the other to pH of 7.6 with tenth normal sodium hydroxide. Then using sterile equipment and aseptic methods 9 c.c. of acid and alkaline urine were each placed in a series of test tubes to which were added 1 c.c. of 1 to 1,000 aqueous solution of the dye. They were then inoculated with one loop of 24 hour broth cultures of Bacillus coli and Staphylococcus aureus. They were incubated for 24 hours after which a loop of each tube of cultured urine was inoculated into a 10 c.c. tube of agar. It was immediately plated and then incubated for 24 hours. At this time the plates were observed for colonies, and recorded in Table I, zero (0) signifies no colonies, few means approximately 25, while infinity (∞) indicates an uncountable number of colonies.

## DETERMINATION OF TOXICITY, EXCRETION AND ANTISEPTIC ACTION OF URINE FOLLOWING INTRAVENOUS INJECTION INTO RABBITS

The dyes that showed antiseptic action in urine were now subjected to further examination. Male rabbits were selected as the animal of choice for this work. A control specimen of urine was obtained from each rabbit just before injection of the dye. This was done by catheterization under aseptic precautions, i.e., catheters were sterilized, a sterile lubricant was used and the external genitalia were washed with a 1 to 10,000 solution of mercury bichloride. Then 1 c.c. of a 1 to 10 aqueous solution for every kilogram of body weight was

injected into the marginal vein of the rabbits ear. (Thus the dosage of the dye was 10 mgms. per kilogram of body weight. In some cases a larger dosage was used, see Table II). Two and four hours after injection, the rabbits were again catheterized and the dye excretion quantitatively estimated (See Table II) by the color of the urine. Bactericidal action of the urine was also determined by taking 1 c.c. samples of each of the above samples of urine and transferring them into sterile test tubes. These were then inoculated with one loop of 24 hour cultures of Staphlococcus aureus and Bacillus coli. After 24 hours of incubation agar plates were made from these samples as previously described. The agar plates were observed after 24 hours of incubation and the results recorded in Table III.

## RESULTS OF EXPERIMENTAL WORK

Table I shows the 35 dyes classified and numbered as far as possible, according to the Colour Index. The results on agar plate and in urine are shown. Those dyes which showed bactericidal or bacteriostatic action were then selected for animal work.

Table II shows the dyes selected for intravenous injection. The dosage used, the renal excretion, as judged by the color of the urine, and the toxic manifestations, if any, are recorded in this table. Three drugs were lethal, namely, victoria blue, night blue and ganus green B. Victoria blue and night blue also showed no evidence of their presence in the urine. It was also doubtful whether or not methylene green, benzo-rhoduline red-B., solid green and ganus green were present in the urine.

Table III shows the antiseptic action of the excreted urine following the intravenous injection of the dye. In the rabbits number, 101, 102, 105, 111, 112, 125 and 128 we find that the control urine showed bacteriolytic action. The favorable results that were recorded in the 2 hour and 4 hour specimen for these dyes will have to be discounted. Of all the dyes used 3-7 - diamino - 10 - methyl-acridinium chloride gave the best results with complete inhibition of both Staphylococcus aureus and Bacillus coli, in the 2 hour specimen in one rabbit and the 4 hour specimen in another. This dye warrants much more careful study. Rhoduline red B, rhoduline red G, hematoxylin, crystal violet resorcinol and acridin - 9 - ethylene also show need for more experimental investigation.

## CONCLUSIONS

1. The requirements, need and limitations of an ideal urinary antiseptic were discussed.

2. The development, merits and present status of each of the following urinary antiseptics, namely, hexamethylenamine, acriflavine, mercurochrome, hexylresorcinol and pyridium were given. Variation in the experimental and clinical results can be noted.

3. The reasons for the investigation of aniline dyes for their antiseptic properties were stated.

4. Of the 35 aniline investigated, 3-7 - diamino - 10 - methyl-acridinium chloride gave the most promising results and should be given more careful experimental analysis.

5. Rhoduline red B, rhoduline red G, hematoxylin, crystal violet resorcinol, and acridine - 9 - ethylene also indicate a need for more work.

TABLE I Antiseptic Properties of Dyes In Agar and Urine

| Group and Name | Number | | Agar Plate Dilution 1:10,000 pH 7.6 | | Urine Media Dilution 1:10,000 pH 6.4 | | | | pH 7.6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Immediate | | 24 hour | | Immediate | | 24 hour | |
| | Colour Index | Schultz | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus |
| Section A - Synthetic Organic Dyestuffs | | | | | | | | | | | | |
| III Mono-Azo | | | | | | | | | | | | |
| Crystal Scarlet | 89 | 113 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Di-phenolamino-Orange ✓ | 143 | 139 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Croceine 3BX | 183 | 167 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Scarlet 6R | 186 | 170 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| IV Dis-Azo | | | | | | | | | | | | |
| (b) Secondary | | | | | | | | | | | | |
| Biebrich Scarlet | 280 | 247 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| (d) From Diamines | | | | | | | | | | | | |
| Naphthol-schwartze (black) | 311 | 269 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Congorot | 370 | 307 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| V Triphenylmethane and Diphenylnaphthylmethane | | | | | | | | | | | | |
| (a) Diamino derivatives of Triphenylmethane Guinea Green | 666 | 502 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| (b) Triamino derivatives of Triphenylmethane Aniline blau | 689 | 521 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Victoria blau * | 690 | 522 | + | 0 | ∞ | ∞ | ∞ | 0 | ∞ | ∞ | ∞ | 0 |
| (e) Derivatives of Diphenyl-naphthylmethane Night blue * | 731 | 560 | + | 0 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| XI Xanthene iii Fluorone (a) Hydroxyphthaleine Uranin (Fluorescein) | 766 | 585 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| XIV Thiazole | | | | | | | | | | | | |
| Thioflavine T ✓* | 815 | 618 | + | 0 | ∞ | ∞ | ∞ | Few | ∞ | ∞ | 0 | 0 |

TABLE I Cont'd.

| Group and Name | | Number | | Agar Plate Dilution 1:10,000 pH 7.6 | | Urine Media Dilution 1:10,000 pH 6.4 | | | | pH 7.6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Immediate | | 24 hour | | Immediate | | 24 hour | |
| | | Colour Index | Schultz | B. Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus |
| XVII Azine | | | | | | | | | | | | | |
| 4.Safranines | | | | | | | | | | | | | |
| (a) Benzosafranines | | | | | | | | | | | | | |
| Rhoduline Red B | * | 844 | 684 | + | 0 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | 0 |
| Rhoduline Red G | * | 844 | 684 | + | 0 | ∞ | ∞ | Few | Few | ∞ | ∞ | ∞ | 0 |
| XX Thiazine | | | | | | | | | | | | | |
| Methylenblau | * | 922 | 659 | + | 0 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | 0 |
| Methylene Green | ✓ * | 924 | 660 | + | 0 | ∞ | ∞ | Few | Few | ∞ | ∞ | ∞ | Few |
| XXIII Anthraquinone | | | | | | | | | | | | | |
| Saure Alizarine Grun | | 1049 | 796 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Saure Alizarine Blau | | 1063 | 790 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| XXVI Indigoid | | | | | | | | | | | | | |
| Indigotine P | * | 1181 | 878 | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | 0 | ∞ |
| Section B-Natural Organic Dyestuffs | | | | | | | | | | | | | |
| Extract of Persian Berry | * | 1234 | 926 | + | + | ∞ | ∞ | 0 | ∞ | ∞ | ∞ | 0 | ∞ |
| Hematoxylin | * | 1246 | 938 | + | 0 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| | | File Number | | | | | | | | | | | |
| Miscellaneous Dyes and Chemicals | | | | | | | | | | | | | |
| Benzo-Rhoduline Red B | ✓ * | 51 M | | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | Few | ∞ |
| Solid Green (O'Plaster) | * | 54 M | | + | + | ∞ | ∞ | Few | ∞ | ∞ | ∞ | ∞ | ∞ |
| Gamus Green B | * | 55 M | | + | 0 | ∞ | ∞ | ∞ | 0 | ∞ | ∞ | Few | 0 |
| Acridine Series from Abbott | | | | | | | | | | | | | |
| | ✓ * | I | | 0 | 0 | ∞ | ∞ | 0 | 0 | ∞ | ∞ | 0 | 0 |
| | ✓ * | II | | 0 | 0 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | 0 | 0 |
| | ✓ * | III | | 0 | 0 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | 0 | 0 |
| | ✓ | IV | | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |

TABLE I Contd

| Group and Name | Number | | Agar Plate Dilution 1:10,000 pH 7.6 | | Urine Media Dilution 1:10,000 pH 6.4 | | | | pH 7.6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Immediate | | 24 hour | | Immediate | | 24 hour. | |
| | Colour Index | Schultz | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus | B.Coli | Staph. Aureus |
| Miscellaneous Dyes and Chemicals Continued:- | File Number | | | | | | | | | | | |
| Crystal Violet Resorcinol * | A | | + | o | ∞ | ∞ | ∞ | o | ∞ | ∞ | o | o |
| 3-7 Diamino-10-methyl-acridinium chloride * | B | | o | o | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | o | o |
| Acridine-9-Eth. * | C | | o | o | ∞ | ∞ | Few | ∞ | ∞ | ∞ | o | o |
| Silver Acriflavine | | | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | o |
| Flumerin | | | + | + | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Acriflavine | | | o | o | ∞ | ∞ | o | o | ∞ | ∞ | o | o |

(V) indicates that the dye was not soluble 1:1000, so saturated solutions were used to add to the media.

(o) means no growth; (∞) means infinite growth of colonies; (+) means a stroke growth.

(*) indicates that the records suggest further investigation of these dyes.

TABLE II

Urinary Excretion of Dyes by Rabbits Following Intravenous Injection

| Dye | Colour Index Number | Schultz Number | Rabbit Number | Dosage Mgm./Kg. | Renal Secretion | Notes |
|---|---|---|---|---|---|---|
| Victoria blue | 690 | 522 | 106 | 10 SS | None | |
| | | | 124 | 8 SS | None | Lethal |
| Night blue | 731 | 560 | 113 | 8 | None | Lethal |
| Thioflavine T | 815 | 618 | 117 | 10 | Slight | |
| Rhoduline Red B | 844 | 684 | 114 | 10 | Moderate | |
| | | | 125 | 10 | Moderate | |
| Rhoduline Red G | 844 | 684 | 115 | 10 | Moderate | |
| Methylenblau | 922 | 659 | 101 | 10 | Slight | |
| | | | 126 | 10 | Slight | |
| Methylene Green | 924 | 660 | 116 | 10 | Doubtful | |
| Indigotine P | 1181 | 878 | 104 | 10 SS | Marked | |
| Ext. of Persian Berry | 1234 | 926 | 102 | 10 | Slight | |
| Hematoxylin | 1246 | 938 | 105 | 10 SS | Moderate | |
| | | | 127 | 25 SS | Marked | |
| Miscellaneous | File No. | | | | | |
| Benzo-Rhodulin Red B | 51 M | | 118 | 10 | Doubtful | |
| Solid Green | 54 M | | 119 | 10 | Doubtful | |
| Ganus Green B | 55 M | | 120 | 10 | Doubtful | Lethal next day. |
| Acridine Series (Abbott) | | | | | | |
| | I | | 109 | 10 SS | Marked | |
| | II | | 110 | 10 SS | Moderate | |
| | III | | 111 | 10 SS | Slight | |
| | | | 128 | 12 SS | Moderate | |
| Crystal Violet Resorcinol | A | | 103 | 10 SS | Moderate | |
| | | | 121 | 10 SS | Slight | |
| 3-7-Diamino-10-methyl-acridinium-chloride | B | | 107 | 10 | Moderate | |
| | | | 122 | 10 | Moderate | |
| Acridine-9-Eth. | C | | 112 | 5 | Moderate | |
| | | | 123 | 20 | Moderate | |

SS means that solutions were saturated in less than 1:100 dilutions.
10 SS means that 10 c.c. per Kg. were used.

## TABLE III

Antiseptic Properties of Rabbits' Urine Following Intravenous Infection of Dyes.

| Dye | Colour Index Number | Schultz Number | Rabbit Number | Control | | 2 Hr. Spec. | | 4 Hr. Spec. | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | B.Coli | Staph | B.C. | S.A. | B.C. | S. A. |
| Victoria blue | 690 | 522 | 106 | ∞ | ∞ | ∞ | ∞ | 0 | 0 |
| Thioflavine T | 815 | 618 | 117 | ∞ | ∞ | ∞ | — | — | — |
| Rhoduline Red B | 844 | 684 | 114 | ∞ | — | ∞ | — | ∞ | 0 |
| | | | 125 | 0 | — | 0 | — | ∞ | 0 |
| Rhoduline Red G | 844 | 684 | 115 | ∞ | ∞ | ∞ | ∞ | ∞ | Few |
| Methylenblau | 922 | 659 | 101 | 0 | ∞ | ∞ | 0 | ∞ | ∞ |
| | | | 126 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Methylene Green | 924 | 660 | 116 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Indigotine P | 1181 | 878 | 104 | ∞ | — | ∞ | ∞ | — | — |
| Ext. of Persian Berry | 1234 | 926 | 102 | 0 | ∞ | ∞ | ∞ | — | — |
| Hematoxylin | 1246 | 938 | 105 | 0 | Few | ∞ | 0 | 0 | 0 |
| | | | 127 | ∞ | ∞ | ∞ | ∞ | ∞ | — |
| Miscellaneous | File No. | | | | | | | | |
| Benzo-Rhoduline Red B | 51 M | | 118 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Solid Green ()'Plaster) | 54 M | | 119 | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| Ganus Green B | 55 M | | 120 | ∞ | — | ∞ | ∞ | — | — |
| Acridin Series (Abbott) | | | | | | | | | |
| | | I | 109 | — | — | — | — | Few | ∞ |
| | | II | 110 | ∞ | ∞ | — | — | ∞ | Few |
| | | III | 111 | 0 | 0 | 0 | Few | 0 | Few |
| | | | 128 | ∞ | 0 | 0 | 0 | ∞ | ∞ |
| Crystal Violet Resorcinol | | A | 103 | ∞ | ∞ | Few | Few | ∞ | ∞ |
| | | | 121 | ∞ | ∞ | ∞ | 0 | ∞ | ∞ |
| 3-7-Diamino-10-methyl-acridinium chloride | | B | 107 | ∞ | — | — | — | 0 | 0 |
| | | | 129 | ∞ | ∞ | 0 | 0 | — | — |
| Acridin-9-Ethylene | | C | 112 | 0 | 0 | ∞ | Few | — | — |
| | | | 123 | ∞ | ∞ | 0 | Few | — | — |

For group refer to Table No. I.

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