

the amount of respiring material. Weighing a half milligram of tissue, even on a semi-micro balance, permits a considerable percentage error; but forcing the tissue into a capillary and measuring its volume gives promise of greater accuracy. That the injury attendant upon the initial dissection is not a constant source of error is shown by the essentially similar values obtained from samples of large and small fragments of the same type of tissue. Such tests likewise indicate an adequate gas diffusion and absorption.

Some actual respiration values obtained on frog blood and nervous tissue, and the reliability of individual readings, are indicated in the tables.

## 13198

### Anti-sulfapyridine and Anti-sulfathiazole Effect of Local Anaesthetics Derived from p-Aminobenzoic Acid.

A. K. KELTCH, LINVILLE A. BAKER, M. E. KRAHL, AND  
G. H. A. CLOWES.

*From the Lilly Research Laboratories, Indianapolis, Indiana.*

It was shown by Woods<sup>1</sup> that the bacteriostatic effect of sulfanilamide or sulfapyridine on hemolytic streptococci *in vitro* could be blocked by small concentrations of p-aminobenzoic acid. Also the diethylaminoethyl ester of p-aminobenzoic acid, used as a local anaesthetic under the names 'Novocaine' or 'Procaine,' was almost as strongly anti-bacteriostatic as p-aminobenzoic acid itself; in concentrations of  $5.8 \times 10^{-8}M$  these compounds could suppress the effect of  $3.03 \times 10^{-4}M$  sulfanilamide.

Owing to the increasing local and systemic use of sulfonamides in treatment of wounds and in surgery where local anaesthetics are concurrently employed, it appeared of interest to determine first, whether or not the anti-bacteriostatic effect of 'Novocaine' could be obtained with other local anaesthetics and, secondly, whether or not local anaesthetics which were not p-aminobenzoic acid derivatives would exhibit an anti-bacteriostatic effect similar to that of 'Novocaine.' To this end, 7 local anaesthetics derived from p-aminobenzoic acid and 9 local anaesthetics not derived from p-aminobenzoic acid have been tested *in vitro* for their ability to

---

<sup>1</sup> Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

## 534 LOCAL ANAESTHETICS ON BACTERIOSTASIS SULFAPYRIDINE

TABLE I.

Effect of 7 Local Anesthetics Derived from p-Aminobenzoic Acid (Nos. 2-8) and 9 Local Anesthetics not Derived from p-Aminobenzoic Acid (Nos. 9-17) on Bacteriostasis of *B. coli* by Sulfapyridine. The Bacterial Population after 24 hr of Growth is Expressed in Terms of Photoelectric Density Readings; a Reading of 100 Represents Approximately 1 Billion Organisms per cc; a Reading of 300 Represents About 6 Billion Organisms per cc.

| Local Anesthetic                                                                   | Conc. local anesthetic<br>moles per l                                             | Growth in various molar conc. of Sulfapyridine |                                 |                          |                        |
|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|------------------------------------------------|---------------------------------|--------------------------|------------------------|
|                                                                                    |                                                                                   | 0                                              | $2 \times 10^{-4}$              | $8 \times 10^{-4}$       | $3.2 \times 10^{-3}$   |
| 1. p-Aminobenzoic Acid<br>(Not an anesthetic—<br>used as control)                  | 0<br>10 <sup>-6</sup><br>10 <sup>-5</sup><br>10 <sup>-4</sup>                     | 356<br><br><br>375                             | 114<br>374<br>351<br><br>348    | 109<br>159<br>337<br>348 | 83<br>91<br>305<br>308 |
| 2. $\beta$ -Diethylaminoethyl-p-amino-<br>benzoate 'Novocaine'                     | 0<br>10 <sup>-5</sup><br>10 <sup>-4</sup><br>10 <sup>-3</sup>                     | 356<br><br><br>383                             | 114<br>251<br>348<br>372        | 109<br>96<br>229<br>359  | 83<br>90<br>109<br>303 |
| 3. $\gamma$ -Dimethylamino- $\alpha$ , $\beta$ -dimethylpropyl-<br>p-aminobenzoate | 0<br>10 <sup>-5</sup><br>10 <sup>-4</sup><br>10 <sup>-3</sup>                     | 356<br><br><br>362                             | 114<br>364<br>355<br>338        | 109<br>133<br>350<br>335 | 83<br>92<br>261<br>343 |
| 4. $\gamma$ -Diethylamino- $\beta$ , $\beta$ -dimethylpropyl-<br>p-aminobenzoate   | 0<br>10 <sup>-5</sup><br>10 <sup>-4</sup><br>10 <sup>-3</sup>                     | 356<br><br><br>362                             | 114<br>113<br>279<br>341        | 109<br>87<br>94<br>268   | 83<br>80<br>78<br>119  |
| 5. $\beta$ -Diethylaminoisohexyl-p-amino-<br>benzoate                              | 0<br>10 <sup>-6</sup><br>10 <sup>-5</sup><br>10 <sup>-4</sup><br>10 <sup>-3</sup> | 378<br><br><br><br>359                         | 107<br>101<br>175<br>334<br>353 | <br><br><br><br>         | <br><br><br><br>       |
| 6. $\gamma$ -Dibutylaminopropyl-p-amino-<br>benzoate                               | 0<br>10 <sup>-5</sup><br>10 <sup>-4</sup><br>10 <sup>-3</sup>                     | 378<br><br><br>353                             | 107<br>106<br>172<br>326        | <br><br><br>             | <br><br><br>           |
| 7. $\beta$ -Dimethylaminoethyl-p-<br>butylaminobenzoate                            | 0<br>10 <sup>-4</sup><br>10 <sup>-3</sup>                                         | 378<br><br>355                                 | 107<br>100<br>176               | <br><br>                 | <br><br>               |
| 8. Ethyl-p-aminobenzoate                                                           | 0<br>10 <sup>-4</sup><br>10 <sup>-3</sup>                                         | 360<br><br>328                                 | 103<br>106<br>147               | <br><br>                 | <br><br>               |
| 9. $\gamma$ -(2-Methyl-piperidino)-propyl-<br>benzoate                             | 0<br>10 <sup>-5</sup><br>10 <sup>-4</sup><br>10 <sup>-3</sup>                     | 356<br><br><br>359                             | 114<br>106<br>106<br>136        | 109<br>86<br>81<br>83    | 83<br>81<br>83<br>80   |
| 10. $\gamma$ -Diethylaminopropyl cinnamate                                         | 0<br>10 <sup>-5</sup><br>10 <sup>-4</sup><br>10 <sup>-3</sup>                     | 356<br><br><br>340                             | 114<br>139<br>115<br>117        | 109<br>88<br>84<br>74    | 83<br>75<br>82<br>62   |

# LOCAL ANAESTHETICS ON BACTERIOSTASIS SULFAPYRIDINE 535

TABLE I. (Continued).

| Local Anesthetic                                                 | Conc. local anesthetic | Growth in various molar conc. of Sulfapyridine |                    |                    |                      |
|------------------------------------------------------------------|------------------------|------------------------------------------------|--------------------|--------------------|----------------------|
|                                                                  |                        | 0                                              | $2 \times 10^{-4}$ | $8 \times 10^{-4}$ | $3.2 \times 10^{-3}$ |
|                                                                  | moles per l            |                                                |                    |                    |                      |
| 11. 2-Butyloxyquinoline carboxylic acid-4-diethylethylenediamide | 0                      | 356                                            | 114                | 109                | 83                   |
|                                                                  | $10^{-5}$              |                                                | 100                | 96                 | 78                   |
|                                                                  | $10^{-4}$              |                                                | 113                | 80                 | 78                   |
|                                                                  | $10^{-3}$              | 358                                            | 106                | 17                 | 22                   |
| 12. Piperidinopropanediol-di-phenylurethane                      | 0                      | 360                                            | 103                |                    |                      |
|                                                                  | $10^{-5}$              |                                                | 94                 |                    |                      |
|                                                                  | $10^{-4}$              |                                                | 106                |                    |                      |
|                                                                  | $10^{-3}$              | 218                                            | 108                |                    |                      |
| 13. Ethenyl-p-diethoxydiphenylamidine                            | 0                      | 378                                            | 107                |                    |                      |
|                                                                  | $10^{-5}$              |                                                | 93                 |                    |                      |
|                                                                  | $10^{-4}$              |                                                | 105                |                    |                      |
|                                                                  | $10^{-3}$              | 383                                            | 68                 |                    |                      |
| 14. 4-Benzoxo-2,2,6-trimethylpiperidine                          | 0                      | 378                                            | 107                |                    |                      |
|                                                                  | $10^{-5}$              |                                                | 100                |                    |                      |
|                                                                  | $10^{-4}$              |                                                | 101                |                    |                      |
|                                                                  | $10^{-3}$              | 357                                            | 102                |                    |                      |
| 15. 2-Benzoxo-2-dimethylamino-methyl-1-dimethylaminobutane       | 0                      | 360                                            | 103                |                    |                      |
|                                                                  | $10^{-5}$              |                                                | 114                |                    |                      |
|                                                                  | $10^{-4}$              |                                                | 96                 |                    |                      |
|                                                                  | $10^{-3}$              | 360                                            | 116                |                    |                      |
| 16. 2-Benzoxo-2-methyl-1-dimethylamino-butane                    | 0                      | 360                                            | 103                |                    |                      |
|                                                                  | $10^{-5}$              |                                                | 113                |                    |                      |
|                                                                  | $10^{-4}$              |                                                | 114                |                    |                      |
|                                                                  | $10^{-3}$              | 348                                            | 111                |                    |                      |
| 17. Cocaine                                                      | 0                      | 360                                            | 107                |                    |                      |
|                                                                  | $10^{-5}$              |                                                | 91                 |                    |                      |
|                                                                  | $10^{-4}$              |                                                | 95                 |                    |                      |
|                                                                  | $10^{-3}$              | 371                                            | 113                |                    |                      |

counteract the bacteriostatic action of sulfapyridine on *B. coli* grown in the simple medium of Kalmanson and Bronfenbrenner.<sup>2</sup>

Three local anaesthetics derived from p-aminobenzoic acid and 3 local anaesthetics not derived from p-aminobenzoic acid were also tested for their ability to counteract the bacteriostatic action of sulfathiazole on *B. coli* in the Kalmanson and Bronfenbrenner medium and of sulfathiazole on *Staphylococcus aureus* grown in an artificial medium. The Lewis strain of *B. coli*, and Strain 679 of *Staphylococcus aureus*, originally obtained from Dr. H. M. Powell, were employed as test organisms.

For the *B. coli* experiments, each culture tube received 2 cc of Kalmanson and Bronfenbrenner's solution A and 2 cc of solution

<sup>2</sup> Kalmanson, G., and Bronfenbrenner, J., *J. Gen. Physiol.*, 1939, **23**, 203.

TABLE II.  
Effect of 3 Local Anesthetics Derived from p-Aminobenzoic Acid (Nos. 2-4) and 3 Local Anesthetics Not Derived from p-Aminobenzoic Acid (Nos. 5-7) on Bacteriostasis of *B. coli* and *Staphylococcus aureus* by Sulfathiazole. The Extent of Growth (24 hr for *B. coli* and 48 hr for *S. aureus*) is expressed as in Table I.

| Local Anesthetic                                                           | Conc. local anesthetic moles per l | Growth of <i>B. coli</i> in various molar conc. of sulfathiazole |                  |                      |                      | Growth of <i>S. aureus</i> in various molar conc. of sulfathiazole |                  |                      |                      |
|----------------------------------------------------------------------------|------------------------------------|------------------------------------------------------------------|------------------|----------------------|----------------------|--------------------------------------------------------------------|------------------|----------------------|----------------------|
|                                                                            |                                    | 10 <sup>-4</sup>                                                 |                  | 8 x 10 <sup>-4</sup> |                      | 10 <sup>-4</sup>                                                   |                  | 4 x 10 <sup>-4</sup> |                      |
|                                                                            |                                    | 0                                                                | 10 <sup>-4</sup> | 0                    | 8 x 10 <sup>-4</sup> | 0                                                                  | 10 <sup>-4</sup> | 0                    | 4 x 10 <sup>-4</sup> |
| None — controls                                                            | 0                                  | 372                                                              | 114              | 108                  |                      | 413                                                                | 70               | 65                   | 62                   |
| 1. p-Aminobenzoic Acid                                                     | 10 <sup>-6</sup>                   |                                                                  | 284              | 126                  |                      |                                                                    | 358              | 87                   | 66                   |
|                                                                            | 10 <sup>-5</sup>                   |                                                                  | 387              | 274                  |                      |                                                                    | 401              | 390                  | 112                  |
|                                                                            | 10 <sup>-4</sup>                   | 365                                                              | 370              | 414                  |                      | 401                                                                |                  | 393                  | 309                  |
| 2. $\beta$ -Diethylaminoethyl-p-aminobenzoate                              | 10 <sup>-5</sup>                   |                                                                  | 109              | 108                  |                      |                                                                    | 395              | 94                   | 48                   |
|                                                                            | 10 <sup>-4</sup>                   |                                                                  | 298              | 120                  |                      |                                                                    | 390              | 424                  | 218                  |
|                                                                            | 10 <sup>-3</sup>                   | 358                                                              | 364              | 291                  |                      | 392                                                                | 404              | 402                  | 420                  |
| 3. $\gamma$ -Dimethylamino- $\alpha,\beta$ -dimethylpropyl-p-aminobenzoate | 10 <sup>-5</sup>                   |                                                                  | 120              | 100                  |                      |                                                                    | 86               | 90                   | 78                   |
|                                                                            | 10 <sup>-4</sup>                   |                                                                  | 263              | 119                  |                      |                                                                    | 410              | 108                  | 64                   |
|                                                                            | 10 <sup>-3</sup>                   | 340                                                              | 360              | 234                  |                      | 400                                                                | 397              | 420                  | 168                  |
| 4. $\gamma$ -Diethylamino- $\beta,\beta$ -dimethylpropyl-p-aminobenzoate   | 10 <sup>-5</sup>                   |                                                                  | 140              | 107                  |                      |                                                                    | 106              | 79                   | 67                   |
|                                                                            | 10 <sup>-4</sup>                   |                                                                  | 122              | 126                  |                      |                                                                    | 429              | 121                  | 72                   |
|                                                                            | 10 <sup>-3</sup>                   | 379                                                              | 284              | 129                  |                      | 412                                                                | 411              | 386                  | 195                  |
| 5. $\gamma$ -(2-methyl-piperidino)-propylbenzoate                          | 10 <sup>-5</sup>                   |                                                                  | 110              | 94                   |                      |                                                                    | 82               | 73                   | 66                   |
|                                                                            | 10 <sup>-4</sup>                   |                                                                  | 97               | 98                   |                      |                                                                    | 77               | 56                   | 60                   |
|                                                                            | 10 <sup>-3</sup>                   | 360                                                              | 80               | 100                  |                      | 391                                                                | 90               | 71                   | 67                   |
| 6. $\gamma$ -Diethylaminopropyl cinnamate                                  | 10 <sup>-5</sup>                   |                                                                  | 121              | 102                  |                      |                                                                    | 85               | 89                   | 70                   |
|                                                                            | 10 <sup>-4</sup>                   |                                                                  | 113              | 110                  |                      |                                                                    | 78               | 81                   | 47                   |
|                                                                            | 10 <sup>-3</sup>                   | 327                                                              | 96               | 102                  |                      | 316                                                                | 67               | 71                   | 57                   |
| 7. 2-Butyloxyquinoline carboxylic acid-4-diethylethylenediamide            | 10 <sup>-5</sup>                   |                                                                  | 116              | 105                  |                      |                                                                    |                  |                      |                      |
|                                                                            | 10 <sup>-4</sup>                   |                                                                  | 114              | 107                  |                      |                                                                    |                  |                      |                      |
|                                                                            | 10 <sup>-3</sup>                   | 350                                                              | 13               | 22                   |                      |                                                                    |                  |                      |                      |

B, followed in order by 2 cc of sulfapyridine solution in sterile distilled water and 1 cc of local anaesthetic solution in sterile distilled water to give the concentrations shown in the tables, 0.4 cc of sterile glucose solution to give a final concentration of 2.5 mg per cc, and sterile distilled water to make 7.9 cc. An inoculum of 0.1 cc ( $5 \times 10^8$  organisms) of 24 hr culture of *B. coli* in the simple medium was added, the inoculated tubes then being incubated at 38°C. Under these conditions, the original inoculum of  $6 \times 10^8$  organisms per cc increased to a maximum of about  $6 \times 10^9$  organisms per cc in 8 to 10 hours, remaining virtually constant thereafter. The extent of growth was estimated, after 24-hour incubation, by photoelectric density readings on an Exton Scopometer, the instrument having been calibrated by making density readings on suspensions of known population. A density reading of 100 corresponds to a count of approximately  $1 \times 10^9$  organisms per cc, a reading of 300 to approximately  $6 \times 10^9$  organisms per cc. Though complete suppression of growth by sulfapyridine can be obtained by use of smaller inocula, the present conditions were deliberately selected to enable sulfapyridine and sulfathiazole to exhibit a pronounced bacteriostatic effect at a concentration of the same order as that which usually prevails in the blood of animals given therapeutic doses of the drug.

For the *Staphylococcus aureus* experiments, the artificial medium contained the following materials per cc: 18 amino acids, in approximately the ratio of hydrolysed casein,<sup>3</sup> to give a total final concentration of 2 mg; glutathione, 100 µg; pantothenic acid, 10 µg; nicotinamide, 50 µg; thiamine, 10 µg;  $\text{Na}_2\text{HPO}_4$ , 1.6 mg;  $\text{MgSO}_4$ ,  $\text{K}_2\text{SO}_4$ ,  $\text{CaCl}_2$ , each 0.16 mg;  $\text{NaCl}$ , 1.6 mg;  $\text{NaHCO}_3$ , 0.8 mg; glucose, 10 mg. The *Staphylococcus aureus*, which had been cultivated on agar slants, was inoculated into beef extract broth and allowed to grow 16 hr at 38°C. This bacterial suspension was then diluted 1:10 with the artificial medium and 0.1 cc of the diluted suspension was transferred to 7.9 cc of the artificial medium under test. This inoculum of approximately  $5 \times 10^5$  organisms per cc increased to a maximum of approximately  $12 \times 10^9$  organisms per cc in the test period of 48 hr.

All the local anaesthetics were commercial samples procured on the open market; they were all used in the form of their hydrochlorides. Where necessary, the conversion of the commercial samples to the pure hydrochlorides was made in this laboratory; the authors are indebted to Dr. E. C. Kleiderer for his collaboration in such purification.

---

<sup>3</sup> Wooley, D. W., and Hutchings, B. L., *J. Bact.*, 1940, **89**, 287.

Typical results with each of 16 local anaesthetics alone and in conjunction with sulfapyridine are shown in Table I. It will be noted that each of the 7 p-aminobenzoic acid derivatives partially or completely blocked the bacteriostatic effect of the sulfapyridine; the blocking effect was reduced but not eliminated by substitution in the amino group or esterification of the carboxyl group of p-aminobenzoic acid. None of the 9 local anaesthetics not derived from p-aminobenzoic acid displayed any antagonism to the sulfapyridine. In these experiments, p-aminobenzoic acid could counteract the effect of approximately 200 to 500 molecular equivalents of sulfapyridine; from Table I the corresponding ratios for local anaesthetics nos. 2, 3, and 4 were, respectively, approximately 20, 40, and 2.

Each of the 3 p-aminobenzoic acid derivatives blocked the action of sulfathiazole; none of the non-p-aminobenzoic acid derivatives displayed any antagonism to sulfathiazole (Table II).

*Summary.* Each of 7 local anaesthetics derived from p-aminobenzoic acid partially or completely blocked the *in vitro* bacteriostatic effect of sulfapyridine upon a strain of *B. coli* grown in a simple medium. None of 9 local anaesthetics not derived from p-aminobenzoic acid displayed any antagonism to the sulfapyridine. Each of 3 local anaesthetics derived from p-aminobenzoic acid blocked the bacteriostatic effect of sulfathiazole on *B. coli* and *Staphylococcus aureus*. None of 3 local anaesthetics not derived from p-aminobenzoic acid displayed any antagonism to the sulfathiazole.

## 13199

### The Assay *Recovery* of Prothrombin Added to Plasma.

JOHN H. FERGUSON.

*From the Department of Pharmacology, University of Michigan, Ann Arbor.*

In the promulgation of current methods of prothrombin assay<sup>1, 2</sup> there has been some investigation of plasma dilutions but no direct experiments to show the ability to *recover* prothrombin added to plasma. This is important in connection with the possibility that

---

<sup>1</sup> Quick, A. J., *J. Am. Med. Assn.*, 1938, **110**, 1658.

<sup>2</sup> Brinkhous, K. M., *Medicine*, 1940, **19**, 329.