COMPARATIVE STUDIES ON THE NATURAL AND ACQUIRED RESISTANCE OF CERTAIN STRAINS OF *ESCHERICHIA COLI* TO THE BACTERIOSTATIC AND GERMICIDAL EFFECTS OF CATIONS¹

EARLE K. BORMAN

Department of Public Service Laboratories, Kentucky Agricultural Experiment Station, Lexington, Kentucky

Received for publication, August 24, 1931

HISTORICAL

The literature is replete with instances demonstrating that an increase in the resistance of a strain of microorganisms to a toxic substance is an adaptation which is frequently attained when successive generations of that strain are subjected to the influence of sublethal or subinhibitory quantities of the toxic substance.

Workers in the early days of specific chemotherapy were lead to postulate the development of drug-fastness in the organisms under study. This conception was first clearly formulated by Mesnil and Nicolle (1906). Ehrlich (1907a, 1907b, 1907c, and 1907d) and his co-workers and Browning (1907, 1908) were the first to demonstrate that the drug-fast character in trypanosomes may be retained in serial animal passages. Uhlenhuth, Hübener and Woithe (1907) were unable to demonstrate this transmissibility in durine infections treated with atoxyl. Mesnil and Brimont (1908a) and Plimmer and Bateman (1908) met with like failure in the treatment of experimental nagana with tartar emetic.

The transmissibility of the drug-fast character *in vivo* is, nevertheless, a general rule. Plimmer and Thomson (1907) encountered it in the treatment of experimental surra and nagana with

¹ Contribution from the Department of Bacteriology of the University of Kentucky.

atoxyl. Mesnil and Brimont (1908b) demonstrated it in a strain of *Trypanosoma evansi* resistant to atoxyl. A further complication of the subject is that the species of experimental animal used plays a large part in the inheritance of the drug-fast character. This has been clearly demonstrated by Breinl and Nierenstein (1908), Moore, Nierenstein and Todd (1908) and Mesnil and Brimont (1908c).

In vivo studies on organisms other than protozoa were first reported by Morgenroth and Kauffmann (1912) who observed the development of resistance to optochin in mice by pneumococci.

A distinct parallelism between resistance *in vivo* and that *in vitro* has been reported by Mesnil and Brimont (1908b) in studies on the use of tartar emetic in experimental surra. Broden and Rodhain (1908), however, in the treatment of human trypanosomiasis noted 1 case in which the resistance of a strain of *Trypanosoma lewisi* to tartar emetic was very great *in vitro* but was not apparent *in vivo*. Ehrlich (1909) noted the exact opposite in 1 case of experimental trypanosomiasis in the mouse. The organisms were highly resistant to partially oxidized arsenophenylglycine *in vivo* but were subnormally resistant *in vitro*. Gonder (1911) found that arsenic-fast strains of *Trypanosoma lewisi* obtained *in vivo* did not lose their resistant character on artificial culture media. The relation between *in vivo* and *in vitro* resistance has been found to be of great theoretical and experimental value by Feirer, Meader and Leonard (1926).

The demonstration *in vitro* of the power of adaptation in bacteria to the unfavorable environment produced by continuous cultivation in a medium containing antiseptics was first made by Kossiakoff (1887). Abbott (1891, 1912) observed an increase in the resistance of *Staphylococcus aureus* to the germicidal action of HgCl_2 by a process of artificial selection. Trambusti (1892) was able to produce an acclimatization to HgCl_2 in several species of organisms. Danysz (1900) produced an arsenic-fast strain of *B. anthracis*. Marks (1910) increased the resistance of a strain of *Salmonella surpestifer* to arsenious acid eight-fold and noted a simultaneous increase in its resistance to antimony of

forty-fold. Altmann and Rauth (1910) reported the development of strains of *E. coli* which were markedly resistant to phenol. Masson (1910) found that the acquired resistance of his organisms to such substances as HgCl_2 , CuSO_4 , resorcinol and salicylic acid was temporary and might at any time be superseded by increased sensitivity.

Regenstein (1912) reported the development of resistance to cresol, saponified solution of cresol and to zinc sulphocarbolate in a strain of *Staphylococcus aureus* which had been grown in a medium containing phenol for several generations. He produced a strain resistant to HgCl_2 and found it to be resistant also to HgBr_2 and $\text{Hg}(\text{CN})_2$. Meader and Feirer (1926) working with AgNO_3 , mercurochrome, formaldehyde and acriflavin found that the drug-fastness obtained in strains of *E. coli*, *Eberthella typhi* and *Aerobacter aerogenes* was highly specific and that its development to one of these substances was often accompanied by an increased sensitivity to the others.

Beginning with the work of Dreser (1893) many workers have demonstrated that the dissociated portion of mineral acids and simple salts thereof is chiefly or perhaps entirely responsible for the effects of these substances on bacterial viability. He found that the inhibiting effect of mercuric salts on yeast could be counteracted by the addition of potassium hyposulphite. He explained this by the fact that the double salt formed does not give free mercuric ions on dissociation, a point well established by the behavior of its solutions in a potential gradient. This idea was in complete accord with the results of Koch (1881), Behring (1890), Heider (1892) and others who showed the direct relationship between rise in temperature and increase in disinfectant power.

The idea first formulated by Dreser has been confirmed and extended by many workers in the field of microbiology. Notable among these are the studies of Paul and Krönig (1896), Krönig and Paul (1897), Scheurlen and Spiro (1897), Bial (1897, 1902), Minervini (1898), Winslow and Lochridge (1906) and Chick (1908, 1910). Clark (1899) working with fungi was forced to disagree in part with this theory but his discordant results can

be partially explained by the fact that he was compelled by the resistant nature of the organisms studied to use solutions which may not strictly be termed dilute. The exact boundary between dilute and concentrated solutions is a debatable point, however, and care should be taken not to define a solution of a mineral acid or a simple salt as concentrated merely because its effects on metabolic processes do not follow the laws of dilute solutions as based on physico-chemical principles.

Of numerous studies on the effect of cations upon bacterial growth those of Eisenberg (1918), Holland (1922) and Hotchkiss (1923) were the first to call attention to the fact that the various cations differ only in degree of potency. They have demonstrated that Na^+ and K^+ , commonly known as favorable to bacterial growth, are markedly toxic in sufficiently high concentrations. Furthermore, these observers have noted that Pb^{++} and Hg^{++} , usually referred to as toxic, are stimulating in sufficiently high dilution, a fact previously noted for the effects of phenol, trikresol, HgCl_2 and arsenicals upon the growth of spirochaetes by Bronfenbrenner and Noguchi (1913). In this connection Shaughnessy and Winslow (1927) have shown that, whereas weak solutions of sodium salts increase the permeability of the bacterial cell wall, stronger solutions decrease its permeability. They have also shown this to be true for solutions of calcium salts although a considerable difference in degree of potency was noted. Moreover, Winslow and Dolloff (1928) have presented results which indicate that the cations in a solution containing a mixture of salts of univalent and bivalent metals exert an additive, not an antagonistic, effect upon bacterial viability. Their analysis of their results is inexact but the technical error involved is not great enough to impair the value of their findings seriously. This matter will be discussed in a later section. More recently Winslow and Haywood (1931) have extended these observations with essentially similar results.

OBJECT OF INVESTIGATION

The present studies were planned to determine in some measure the answers to the following questions:

1. Do the relative germicidal potencies of various cations follow each other in the same ascending order as the relative bacteriostatic potencies for each strain of the organism under observation when determined under identical conditions?

2. Does the development of increased resistance to one or more cations affect in any manner the degree of resistance of the same organism to other cations?

EXPERIMENTAL

Since it was desirable to compare the results of this investigation with the work of previous investigators in this field, *Escherichia coli* was chosen as the test organism. Two strains were used. Strain A was isolated from a contaminated water supply. Strain B, kindly furnished by Dr. C.-E. A. Winslow of Yale University, was the strain used by Hotchkiss (1923), by Winslow and Dolloff (1928), by Winslow and Haywood (1931) and by others working in the same laboratory.

All salts used were chlorides of the highest purity obtainable. All glassware used in these studies was carefully cleaned by placing it in dichromate cleaning solution for twenty-four hours with a subsequent series of four rinsings in warm distilled water.

The following media were used in determining the critical bacteriostatic concentrations of the salts:

1. The peptone solution, used by Hotchkiss (1923) and by Winslow and Dolloff (1928), consisting of 1 per cent Difco bacto-peptone in distilled water.

2. Dolloff's synthetic medium, used by Winslow and Dolloff (1928), consisting of 5 grams Pfanstiehl c. p. lactose, 5 grams recrystallized ammonium tartrate and 0.02 gram dibasic ammonium phosphate in 1,000 cc. distilled water.

The critical bacteriostatic concentrations of the salts were determined upon a test suspension of the organism consisting of a twenty-four hour culture in either of the above media which had been incubated at 37°C. Inoculations of 0.05 cc. were made into tubes containing 5 cc. homologous medium and varying concentrations of the salt in question. These subcultures were incubated at 37°C. for forty-eight hours. The tubes were then

examined for signs of growth. The *critical bacteriostatic concentration* was taken as the lowest concentration expressed as molality of the cation which showed complete inhibition of growth. The tube containing the highest concentration of salt showing growth was in each case streaked on Endo's medium for confirmation. All determinations were made in triplicate.

The germicidal efficiency of the various salts was determined at 37°C. The test suspension of the organism was prepared by centrifugalizing a twenty-four hour culture of the organism in Dolloff's medium and washing the sedimented bacterial cells three times by resuspending them in sterile distilled water with subsequent centrifugalization. The sedimented organisms were then suspended in sterile distilled water and the turbidity adjusted to match that of a standard suspension of BaSO₄ arbitrarily chosen and roughly approximating the density of an eighteen-hour culture of *E. coli* in Dolloff's medium. The bacterial suspension was warmed to 37°C. and used immediately. The salt solutions to be tested were made up in varying concentrations under aseptic conditions and 2.5 cc. of each concentration were placed in sterile test tubes provided with a cotton plug. These solutions were then warmed to 37°C. An inoculum of 0.25 cc. of the test suspension was placed in each tube and the whole shaken and replaced in the water bath at 37°C. An exposure of six hours was found to be the most satisfactory from the standpoint of greatest accuracy over the range of concentrations used. Subcultures were made by transferring one loopful of the mixture into double strength nutrient broth containing 0.5 per cent lactose. A platinum loop 4 mm. in diameter was used. These subcultures were then incubated at 37°C. for forty-eight hours and examined for signs of growth. All growth suspected of being due to contamination was streaked on Endo's medium for confirmation. The lowest concentration expressed as molality of the cation which would kill the test organism under the experimental conditions was regarded as the *critical germicidal concentration* for the time of exposure. The results were all checked by two or more separate determinations.

The resistance of each strain to two cations, Mg⁺⁺ and Hg⁺⁺,

was then increased by growing the organism in successive transplants in Dolloff's medium containing the highest concentration of the salt in question which would permit growth. From time to time this concentration could be increased until an apparent maximum was reached. The resistance of strain A to Mg^{++} was increased four-fold after 84 serial transfers; to Hg^{++} , thirty-fold after 79 serial transfers. The resistance of strain B to Mg^{++} increased three-fold after 90 transfers; to Hg^{++} , twenty-fold after 85 transfers.

TABLE 1

The critical bacteriostatic concentrations for strain A

CATION	MENSTRUUM	MOLALITY OF SALT
K^{+}	Dolloff's medium	0.75
	Peptone medium	1.00
Na^{+}	Dolloff's medium	0.70
	Peptone medium	1.00
Mg^{++}	Dolloff's medium	0.18
	Peptone medium	0.25
Ba^{++}	Peptone medium	0.18
Ca^{++}	Peptone medium	0.16
Fe^{+++}	Dolloff's medium	0.00025
	Peptone medium	0.001
Cu^{++}	Dolloff's medium	0.0005
	Peptone medium	0.0006
Pb^{++}	Peptone medium	0.0004
Hg^{++}	Dolloff's medium	0.00000025
	Peptone medium	0.00000075

These resistant substrains were then tested as before for the bacteriostatic and germicidal effects of the various cations upon them. The test suspensions in this case, however, were prepared from a twenty-four-hour culture in Dolloff's medium containing that concentration of the salt to which the organism's resistance had been enhanced.

The results obtained are recorded in tables 1 to 6. Table 2 contains the data of Winslow and Dolloff (1928) and of Hotchkiss (1923) upon the same organism.

TABLE 2
Critical bacteriostatic concentrations for strain B

CATION	OBSERVER	MENSTRUUM	MOLALITY OF SALT
K ⁺	Borman	Dolloff's medium	0.80
	Winslow and Dolloff	Dolloff's medium	0.80
	Borman	Peptone medium	0.95
	Winslow and Dolloff	Peptone medium	1.00
	Hotchkiss	Peptone medium	2.00
Na ⁺	Borman	Dolloff's medium	0.70
	Winslow and Dolloff	Dolloff's medium	0.60
	Borman	Peptone medium	0.90
	Hotchkiss	Peptone medium	2.00
Mg ⁺⁺	Borman	Dolloff's medium	0.20
	Winslow and Dolloff	Dolloff's medium	0.20
	Borman	Peptone medium	0.30
	Hotchkiss	Peptone medium	0.50
Ba ⁺⁺	Borman	Peptone medium	0.20
	Hotchkiss	Peptone medium	0.25
	Winslow and Dolloff	Asparagin medium	0.20
Ca ⁺⁺	Borman	Peptone medium	0.18
	Winslow and Dolloff	Peptone medium	0.20
	Hotchkiss	Peptone medium	0.50
Fe ⁺⁺⁺	Borman	Dolloff's medium	0.0005
	Borman	Peptone medium	0.0005
	Hotchkiss	Peptone medium	0.001
Cu ⁺⁺	Borman	Dolloff's medium	0.00075
	Borman	Peptone medium	0.001
	Hotchkiss	Peptone medium	0.001
Pb ⁺⁺	Borman	Peptone medium	0.0005
	Hotchkiss	Peptone medium	0.0005
	Winslow and Dolloff	Asparagin medium	0.0005
Hg ⁺⁺	Borman	Dolloff's medium	0.000005
	Borman	Peptone medium	0.000001
	Winslow and Dolloff	Peptone medium	0.000005
	Hotchkiss	Peptone medium	0.000001

THEORETICAL DISCUSSION

The medium described by Dolloff (1926) furnishes an admirable menstruum for the study of salt action. Its relatively simple composition assures uniform results with several lots of media. This uniformity is a potent factor in an investigation so consum-

TABLE 3

Critical bacteriostatic concentrations for resistant substrains of strain A in Dolloff's medium

(Concentration expressed as molality of salts)

CATION	STRAIN A	RESISTANT SUBSTRAIN	
		Mg ⁺⁺	Hg ⁺⁺
K ⁺	0.75	1.50	2.10
Na ⁺	0.70	1.50	2.00
Mg ⁺⁺	0.18	0.72	0.90
Fe ⁺⁺⁺	0.00025	0.0005	0.001
Cu ⁺⁺	0.0005	0.001	0.005
Hg ⁺⁺	0.0000025	0.000005	0.000075

TABLE 4

Critical bacteriostatic concentrations for resistant substrains of strain B in Dolloff's medium

(Concentration expressed as molality of salts)

CATION	STRAIN B	RESISTANT SUBSTRAINS	
		Mg ⁺⁺	Hg ⁺⁺
K ⁺	0.80	1.25	2.20
Na ⁺	0.70	1.25	2.00
Mg ⁺⁺	0.20	0.60	0.80
Fe ⁺⁺⁺	0.0005	0.00075	0.00125
Cu ⁺⁺	0.00075	0.002	0.003
Hg ⁺⁺	0.000005	0.000005	0.00001

ing of time and media. This medium affords a heavy growth of *E. coli* and, incidentally, of *Aerobacter aerogenes* which shows no tendency to become less dense in continuous serial transfers. Its main disadvantage lies in the fact that it is chemically incompatible with certain salts, such as CaCl₂.

The practice adopted herein of expressing cation concentration

in terms of molality is not technically correct and has no real quantitative significance. This practice has been adhered to, however, because of the lack of a better one. Winslow and Dolloff (1928) have adopted the use of the ionic activity. Their

TABLE 5
Critical germicidal concentrations (six hours' exposure) for strain A and its resistant substrains
(Concentration expressed as molality of salt)

CATION	STRAIN A	RESISTANT SUBSTRAINS	
		Mg ⁺⁺	Hg ⁺⁺
Na ⁺	4.00	4.50	>6.15*
Mg ⁺⁺	1.30	2.00	2.30
Ba ⁺⁺	1.10	>1.6*	>1.6*
Ca ⁺⁺	1.25	2.10	2.50
Fe ⁺⁺⁺	0.0002	0.001	0.008
Cu ⁺⁺	0.002	0.008	0.0125
Hg ⁺⁺	0.0000005	0.0000002	0.0000005

* Saturated solution does not kill.

TABLE 6
Critical germicidal concentrations (six hours, exposure) for strain B and its resistant substrains
(Concentration expressed as molality of salt)

CATION	STRAIN B	RESISTANT SUBSTRAINS	
		Mg ⁺⁺	Hg ⁺⁺
Na ⁺	4.20	4.50	>6.15*
Mg ⁺⁺	1.75	2.80	3.20
Ba ⁺⁺	1.20	>1.6*	>1.6*
Ca ⁺⁺	1.75	2.70	3.20
Fe ⁺⁺⁺	0.0005	0.001	0.01
Cu ⁺⁺	0.0025	0.01	0.025
Hg ⁺⁺	0.0000001	0.0000008	0.0000002

* Saturated solution does not kill.

use of this term, however, has been very loose. Since the activity coefficient of an individual ion is dependent upon the total ionic strength of the solution, it would become necessary to know the exact concentration and valence of each ion in the menstruum

employed. This is not always readily ascertainable. The calculation of the ionic strength in Dolloff's medium is complicated by the fact that certain cations form complexes with the ammonium ion and by the partial conversion of the carbohydrate to acid by the heat of sterilization. Finally, the inadequacy of the data available for the determination of ionic activities renders the use of this term impracticable for application to biological studies over more than a limited range of concentrations.

In this connection it is pertinent to note that Winslow and Dolloff (1928) have apparently confused molality with ionic strength. Whereas these two terms are equivalent for solutions of salts containing two univalent ions, this is not true of the solutions of any salt containing a bivalent ion. Reference to the Lewis and Randall (1923) tables shows the values for the activity of Mg^{++} as computed by Winslow and Dolloff to be erroneously derived. The magnitude of this error is not, however, great enough to render their conclusions unacceptable.

ANALYSIS OF TABULATED DATA

Before attempting to correlate the results obtained it is fitting to call attention to the remarkable agreement of the results of three investigations on the critical bacteriostatic concentrations for strain B. The results of this investigation are, with a few exceptions, in remarkable accord with the results of Hotchkiss and of Winslow and Dolloff. Hotchkiss's high values are partially explained by her use of a peptone medium which in our hands yielded values somewhat higher than those obtained with Dolloff's medium.

An analysis of the relative bacteriostatic potencies of the cations shows that for each of the strains of *E. coli* studied they can be arranged in the following ascending order: K^+ and Na^+ ; Ca^{++} , Mg^{++} and Ba^{++} ; Cu^{++} , Fe^{+++} and Pb^{+++} ; Hg^{++} . This confirms the findings of Hotchkiss (1923) and of Eisenberg (1918) with a slight relative variation in the placing of Cu^{++} , Fe^{+++} and Pb^{+++} .

The relative germicidal potencies follow in the same order for those so tested. An acquired increase in the resistance to the

bacteriostatic action of Mg^{++} and of Hg^{++} induced comparable increases in this resistance to the other cations with each strain. This increased resistance was accompanied by a parallel rise in resistance to the germicidal action of all the cations tested. This non-specific stimulation of the mechanism of salt-resistance is not necessarily in disagreement with the results of Meader and Feirer (1926). It may be regarded as a group specificity which would not have been detected in such a diversified series of anti-septics as were employed by these authors. Attention is again called to the results of Marks (1910) who, by increasing the resistance of *Salmonella suispestifer* to arsenic, increased by an even greater quantity its resistance to antimony.

GENERAL CONCLUSIONS

Under the experimental conditions involved in so far as they apply to the specific cations and organisms studied the following conclusions are valid:

1. The relative bacteriostatic and germicidal potencies of cations follow each other in the same ascending series for each strain of the organism.
2. The germicidal potency of any cation occupies the same relative position in a series of such potencies as does the bacteriostatic potency in its homologous series.
3. Acquired resistance to the bacteriostatic action of one cation induces a comparable increase in resistance to the bacteriostatic and germicidal action of other cations with different chemical properties.

These considerations suggest that both the bacteriostatic and the germicidal effects of cations are produced by identical or intimately related properties, probably physico-chemical in nature. Furthermore, they indicate that the defense mechanism invoked by the organism undergoing artificial stimulation of resistance to bacteriostatic and germicidal power is the same for all cations.

Assuming the theory of disinfection postulated by Bancroft and Richter (1931) to be true, these studies indicate that all cations function in the same manner in producing precipitation of the emulsoid colloids of the bacterial cell. It is possible that the

newer knowledge of the effects of cations upon bacteria may form a working basis for the development of a general law covering the effects of salts upon emulsoid sols in general.

REFERENCES

- ABBOTT, A. C. 1891 Corrosive sublimate as a disinfectant against the *Staphylococcus pyogenes aureus*. Bull. Johns Hopkins Hosp., **2**, 50.
- ABBOTT, A. C. 1912 On induced variations in bacterial functions: an experimental study. Jour. Med. Res., **26**, 513.
- ALTMANN, K., AND RAUTH, A. 1910 Experimentelle Studien über Erzeugung serologisch nachweisbarer Variationen beim *Bacterium coli*. Ztschr. f. Immunitätsforsch. u. exper. Therap., O., **7**, 629.
- BANCROFT, W. D., AND RICHTER, G. H. 1931 The chemistry of disinfection. Jour. Phys. Chem., **35**, 511.
- BEHRING, E. A. VON 1890 Ueber Desinfektion, Desinfektionsmittel und Desinfektionsmethoden. Ztschr. f. Hyg., **9**, 395.
- BIAL, M. 1897 Ueber den Mechanismus der Gasgährungen im Magensaft. Arch. f. exper. Path. u. Pharmacol., **33**, 1.
- BIAL, M. 1902 Ueber die antiseptische Funktion des H-Ions verdünnter Säuren. Ztschr. f. phys. Chemie, **40**, 513.
- BREINL, A., AND NIERENSTEIN, M. 1908 Weitere Beobachtungen über Atoxyfestigkeit der Trypanosomen. Deutsch. med. Wchnschr., **34**, 1181.
- BRODEN, A., AND RODHAIN, J. 1908 Traitement de la trypanosomiase humaine. Arch. f. Schiffs- u. Tropen-Hyg., **12**, 443.
- BRONFENBRENNER, J., AND NOGUCHI, H. 1913 On the resistance of various spirochaetes in cultures to the action of chemical and physical agents. Jour. Pharmacol. and Exper. Therap., **4**, 332.
- BROWNING, C. H. 1907 Experimental chemotherapy in trypanosome infections. Brit. Med. Jour., **2**, 1405.
- BROWNING, C. H. 1908 Chemotherapy in trypanosome infections: an experimental study. Jour. Path. and Bact., **12**, 166.
- CHICK, HARRIETTE 1908 An investigation of the laws of disinfection. Jour. Hyg., **8**, 92.
- CHICK, HARRIETTE 1910 The process of disinfection by chemicals and hot water. Jour. Hyg., **10**, 237.
- CLARK, J. F. 1899 On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gazette, **28**, 289; also Jour. Phys. Chem., **3**, 203.
- DANYSZ, J. 1900 Immunisation de la Bactériémie charbonneuse contre l'action du serum du rat. Ann. de l'Inst. Past., **14**, 641.
- DOLLOFF, A. F. 1926 A simple synthetic medium for cultivation of bacteria. Science, **64**, 254.
- DRESER, H. 1893 Zur Pharmacologie des Quecksilbers. Arch. f. exper. Path. u. Pharmacol., **32**, 456.
- EHRlich, P. 1907a Chemotherapeutische Trypanosomen-Studien. Berlin. klin. Wchnschr., **44**, 233.

- EHRlich, P. 1907b Chemotherapeutische Trypanosomen-Studien. Berlin. klin. Wehnschr., **44**, 280.
- EHRlich, P. 1907c Chemotherapeutische Trypanosomen-Studien. Berlin. klin. Wehnschr., **44**, 310.
- EHRlich, P. 1907d Chemotherapeutische Trypanosomen-Studien. Berlin. klin. Wehnschr., **44**, 341.
- EHRlich, P. 1909 Ueber die neuesten Ergebnisse auf dem Gebiete der Trypanosomenforschung. Arch. f. Schiffs- u. Tropen-Hyg., **13**, 91.
- EISENBERG, P. 1918 Untersuchungen über spezifische Desinfektionsvorgänge. II. Mitth. über die Wirkung von Salzen und Ionen auf Bakterien. Centralbl. f. Bakt., I., O., **82**, 69.
- FEIRER, WM. A., MEADER, PERCY D., AND LEONARD, VEADER. 1926 Development of the drug-fast character in vitro and its bearing upon drug rotation in the management of chronic urinary conditions. Jour. Urol., **16**, 97.
- GONDER, RICHARD 1911 Untersuchungen über arzneifeste Mikroorganismen. I. *Trypanosoma lewisi*. Centralbl. f. Bakt., I. O., **61**, 102.
- HEIDER, A. 1892 Ueber die Wirksamkeit der Desinfektionsmittel bei erhöhter Temperatur. Arch. f. Hyg., **15**, 341.
- HOLLAND, D. F. 1922 The effect of potassium and magnesium chlorides on the viability and electrical resistance of *Bacterium coli communis*. Diss., Yale Univ. Library; cited by Winslow and Dolloff (1928).
- HOTCHKISS, MARGARET 1923 The stimulating and inhibitive effect of certain cations upon bacterial growth. Jour. Bact., **8**, 141.
- KOCH, ROBERT 1881 Ueber Desinfektion. Mitth. a. d. kaiserlichen Gsndhtsamte, **1**, 1.
- KOSSIAKOFF, G. 1887 De la propriété que possèdent les microbes de s'accommoder aux milieux antiseptiques. Ann. de l'Inst. Past., **1**, 465.
- KRÖNIG, B., AND PAUL, TH. 1897 Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfektion. Ztschr. f. Hyg., **25**, 1.
- LEWIS, G. N., AND RANDALL, M. 1923 Thermodynamics and the free energy of chemical substances. New York.
- MARKS, LEWIS H. 1910 Ueber einen arsenfesten Bakterienstamm. Ztschr. f. Immunitätsforsch. u. exper. Therap., O, **7**, 293.
- MASSON, LOUIS 1910 Sur l'accoutumance des bactéries aux antiseptiques. Compt. rend. de l'Acad. des Sciences, **150**, 189.
- MEADER, PERCY D., AND FEIRER, WM. A. 1926 Drug-fastness in its relation to the resistance of certain organisms toward familiar germicides. Jour. Inf. Dis., **39**, 237.
- MESNIL, F., AND BRIMONT, E. 1908a Sur une race de trypanosomes résistante à l'émetique et sur l'évaluation in vitro de sa résistance. Compt. rend. de la Soc. de Biol., **14**, 820.
- MESNIL, F., AND BRIMONT, E. 1908b Sur les propriétés de races de trypanosomes résistantes à l'atoxyl et aux sérums. Compt. rend. de la Soc. de Biol., **14**, 637.
- MESNIL, F., AND BRIMONT, E. 1908c Sur les propriétés des races de trypanosomes résistantes aux médicaments. Ann. de l'Inst. Past., **22**, 856.
- MESNIL, F., AND NICOLLE, M. 1906 Traitement des trypanosomiasés par les "couleurs de benzidine." Ann. de l'Inst. Past., **20**, 513.

- MINERVINI, R. 1898 Ueber die baktericide Wirkung des Alkohols. *Ztschr. f. Hyg.*, **29**, 117.
- MOORE, B., NIERENSTEIN, M., AND TODD, J. L. 1908 Concerning the treatment of experimental trypanosomiasis. *Ann. Trop. Med. and Parasitol.*, **2**, 265.
- MORGENROTH, J., AND KAUFFMANN, M. 1912 Arzneifestigkeit bei Bakterien (Pneumokokken). *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, I., O., **15**, 610.
- PAUL, TH., AND KRÖNIG, B. 1896 Ueber das Verhalten der Bakterien zu chemischen Reagentien. *Ztschr. f. phys. Chemie*, **21**, 414.
- PLIMMER, H. G., AND BATEMAN, H. R. 1908 Further results of the experimental treatment of trypanosomiasis: progress report. *Proc. Roy. Soc.*, **80**, 477.
- PLIMMER, H. G., AND THOMSON, J. D. 1907 A preliminary summary of the results of the experimental treatment of trypanosomiasis in rats. *Proc. Roy. Soc.*, **79**, 505.
- REGENSTEIN, HANS 1912 Studien über die Anpassung von Bakterien an Desinfektionsmittel. *Centralbl. f. Bakt.*, I., O., **63**, 281.
- SCHOURLEN AND SPIRO 1897 Die gesetzmässigen Beziehungen zwischen Lösungszustand und Wirkungswert der Desinfektionsmittel. *Münch. med. Wchnschr.*, **44**, 81.
- SHAUGHNESSY, H. J., AND WINSLOW, C.-E. A. 1927 The diffusion products of bacterial cells as influenced by the presence of various electrolytes. *Jour. Bact.*, **14**, 69.
- TRAMBUSTI, A. 1892 Contributo sperimentale alla legge del l'adattamento dei microorganismi ai mezzi antisettici. *Lo Sperimentale*, **1**, 29. *Ref. Centralbl. f. Bakt.*, **13**, 673.
- UHLENHUTH, P., HÜBENER AND WOITHE 1907 Experimentelle Untersuchungen von Dourins mit besonderer Berücksichtigung der Atoxylbehandlung. *Arb. a. d. kaiserlich. Gsndtsamte.*, **27**, 256.
- WINSLOW, C.-E. A., AND DOLLOFF, A. F. 1928 Relative importance of additive and antagonistic effects of cations upon bacterial viability. *Jour. Bact.*, **15**, 67.
- WINSLOW, C.-E. A., AND HAYWOOD, ELOISE T. 1931 The specific potency of certain cations with reference to their effect on bacterial viability. *Jour. Bact.*, **22**, 49.
- WINSLOW, C.-E. A., AND LOCHRIDGE, E. E. 1906 The toxic effect of certain acids upon colon and typhoid bacilli in relation to their degree of dissociation. *Jour. Inf. Dis.*, **3**, 547.