13804

Evaluation of Germicides with Relation to Tissue Toxicity.

MARVIN M. HIRSCH* AND MILAN V. NOVAK.

From the Department of Bacteriology and Public Health, University of Illinois.

The consensus of opinion of investigators and clinicians is that tissue toxicity of germicides should be evaluated as well as their germicidal power. Tissue culture,[1-4] manometric,[5,6] and phagocytosis inhibition[7,8,9] studies have been made. The skin and blood, the primary barriers to infection, are tissues most affected by the use of germicides. From a clinical standpoint, therefore, the criterion of tissue toxicity should be based on the destruction of these two tissues. Nye,[7] Welch and Hunter,[8] and Welch and Brewer[9] tested germicides, using inhibition of phagocytosis as the criterion of toxicity.

The present study was concerned with an attempt to standardize certain factors in the phagocytosis inhibition technic with regard to time relationships and the quality and quantity of organic matter entering into the reaction. A test organism was used which needed no artificial opsonization and was comparatively resistant to the action of germicides in general. An attempt was also made to establish the relative sensitivity of skin and blood to the destructive effects of germicides.

The so-called Toxicity Index has been described as toxicity end point/germicidal end point.[4-9] This same ratio was used in the present study. The toxicity end point was the dilution of antiseptic completely inhibiting phagocytosis. The germicidal end point was the dilution completely killing the test organism in the presence of blood.

---

* Portion of thesis submitted in partial fulfillment of the requirements for the degree Master of Science in the Graduate School of the University of Illinois.

1 Lambert, R. A., and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.

2 German, W. M., *Arch. Surg.*, 1928, **18**, 1920.

3 Buchsbaum, R., and Bloom, W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1060.

4 Salle, A. I., McOmie, W. A., Schechmeister, I. L., and Foord, D. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 694.

5 Bronfenbrenner, J., Hershey, A. D., and Doubly, J. A., *J. Bact.*, 1939, **37**, 583.

6 Ely, J. D., *J. Bact.*, 1939, **38**, 391.

7 Nye, R. N., *J. A. M. A.*, 1937, **108**, 280.

8 Welch, H., and Hunter, A. C., *Am. J. Pub. Health*, 1940, **30**, 129.

9 Welch, H., and Brewer, C. M., *J. Immunol.*, 1942, **43**, 25.

The toxicity end point was determined as follows: Four-tenths ml of decreasing concentrations of the germicide (diluted with 0.85% NaCl) was placed into a series of 1 x 10 cm test tubes in a water bath at 37°C. Four-tenths ml fresh human whole blood, containing 0.25% sodium citrate, was added and the final dilution of the germicide was calculated. After 30 minutes' incubation at 37°C, during which time the tubes were frequently shaken, 0.2 ml of a standardized suspension of the test organism was added. The bacterial suspension consisted of 240-270 million organisms per ml in 0.85% NaCl, from a washed 24-hour nutrient agar culture of a phagocytable strain of *Staphylococcus albus*, isolated from the air and found to ferment mannitol but not to coagulate plasma. After addition of the bacteria, the contents of the tubes were mixed at 37°C in a device which simultaneously rotated the tubes and tipped them end to end for a period of 5-10 minutes in order to assure intimate contact between leukocytes and bacteria. A smear from each tube was stained with Wright's stain. After examination of 25 polymorphonuclear leukocytes, the slide, representing the highest dilution of the germicide, which shows an average of less than one engulfed bacterium per cell was considered as the toxicity end point. Controls with 0.85% NaCl in place of the germicide were used to show complete phagocytosis under the test conditions.

The germicidal end point was determined by placing 0.4 ml of decreasing concentrations of the germicide in a series of 1 x 10 cm sterile test tubes at 37°C to which were added 0.4 ml of citrated human blood (old enough to contain no living leukocytes) and 0.2 ml of the bacterial suspension as described above. The final dilution of the germicide was noted. After 30 minutes' incubation at 37°C, one 4 mm loopful from each tube was transferred to broth. A second transfer of four 4 mm loopfuls was made to avoid bacteriostatic effects. The transfers were incubated at 37°C for 48 hours. The highest dilution of the germicide showing no growth was taken as the germicidal end point. Positive controls using bacteria and blood, and sterile controls consisting of blood and saline were used.

The results are summarized in Table I. A toxicity index of less than one indicates a germicide, whose lowest germicidal concentration is non-toxic. On the other hand a value of more than one indicates relative increased toxicity of the chemical to phagocytosis. Four compounds (in aqueous solution only) display toxicity indices of less than one, with efficiency ranges as follows: mercuric chloride, 1:1030-1:1730; N-N'-dichloroazocarbonamide, 1:3000-1:5000; alkyl-dimethyl-benzyl-ammonium chloride, 1:3370-1:3970; and 4 ni-

TABLE I.
Order of Efficiency of 24 Germicides as Measured by the Toxicity Index.

		T.	G.	T.I.
1.	Mercuric chloride	1:1030	1:1730	0.59
2.	N.-N1-dichloroazocarbonamide	1:3000	1:5000	0.60
3.	Alkyl-dimethyl-benzyl-ammonium chloride (aqueous)	1:3370	1:3970	0.85
4	4-nitro-5-hydroxymercuri-o-cresol (aqueous)	1:1330	1:1400	0.95
5.	Phenyl mercuric nitrate	1:2500*	1:2100*	1.2
6.	2-furylmercuric hydroxide (aqueous)	1:5700*	1:3700*	1.5
7.	2-furylmercuric hydroxide (tincture)	1:8000	1:5000	1.6
8.	Phenol	1:100	1:63	1.6
9.	Iodine (aqueous)	1:200	1:120	1.7
10.	4-nitro-5-hydroxymercuri-o-cresol (tincture)	1:3400	1:1570	2.2
11.	Iodine (tincture)	1:470	1:180	2.6
12.	Alkyl-dimethyl-benzyl-ammonium chloride (tincture)	1:19,000	1:7000	2.7
13.	1,3 hydroxy-4-hexylbenzene	1:4000	1:1400*	2.8
14.	Tinc. cresol and mercuric chloride	1:12	1:4	3.0
15.	Tri-cresol	1:800	1:200	4.0
16.	50% alcohol—10% acetone	1:13	1:3.3	4.0
17.	Alcohol 95%	1:17	1:3.7	
				Av. 5.2
18.	Alcohol 70%	1:27	1:4.7	
19.	Sodium-ethyl-mercurithiosalycilate (tincture)	1:17,700	1:2300	7.7
20.	Disodium 2,7 dibromo-4-hydroximercuri-fleuroescein (aqueous)	1:630	1:55*	11.5
21.	Disodium 2,7, dibromo-4-hydroximercuri-fleuroescein (tincture)	1:5300	1:430	12.5
22.	Sodium-ethyl-mercurithiosalycilate (aqueous)	1:1470	1:100	14.7
23.	Sodium hypochlorite	1:20	1:1*	20
24.	Tincture Green Soap	1:1830	1:2	900

*An excess of germicide in original concentration was required to obtain these results.

T = Toxic End Point. Highest dilution completely inhibiting phagocytosis.

G = Germicidal End Point. Highest dilution completely sterilizing infected blood.

T.I. = Toxicity Index = T/G.

tro-5-hydroxymercuri-o-cresol, 1:1330-1:1400. The efficiency range is regarded as the limits of concentration within which a germicide, under the conditions of the test, will completely destroy bacteria but will not destroy tissue. A substance with a toxicity index greater than one has no such efficiency range.

Further perusal of Table I indicates that the chlorine compounds rate the highest while the organic mercurials rate among the poorest, although this is not a strict rule. The phenolic compounds hold a more or less intermediate position.

No tincture preparation shows a toxicity index of less than one in this study. This would seem to indicate that the solvent itself is more toxic to phagocytosis than to bacteria. This is borne out by the results for the common tincture solvents, 50% alcohol-10% acetone and 70% alcohol.

Guinea pigs were used to determine the relative sensitivity of skin

and blood to the action of germicides. Areas of 1 sq cm on the shaved skin were scarified. The various germicides were applied constantly (to prevent evaporation) to cotton compresses on the scarified areas. Saline solution was used on control areas. Intraperitoneal injections of 1 ml of 15% urethane per 100 g of body weight were used to keep the animals under constant anesthesia. Results show that of all germicides tested the highest dilution, previously found to completely inhibit phagocytosis, manifested no toxic reaction to scarified skin for equal periods of exposure.

Discussion. A method standardizing certain factors in the phagocytosis inhibition technic of rating germicides is proposed. In previous studies[8, 9] the germicidal power was measured by incubating a mixture of germicide, blood, and bacteria for 30 minutes. The same principle was applied in the present study. The toxic power in the previous studies was determined by 30 minutes' incubation of a mixture of germicide, fresh blood, and opsonized bacteria. This in reality measured the instantaneous inhibition of phagocytosis. The present study, however, measured toxicity by first incubating the germicides and fresh blood for 30 minutes and then adding the bacteria. This standardized the time relationships of the toxicity and germicidal tests.

The previous studies made use of an artificially opsonized saline suspension of the antigen for the toxicity tests and a broth-culture suspension for the germicidal tests. In the present study the test organism, which need not be artificially opsonized, was used in saline suspension for both the toxicity and the germicidal tests. This standardized the amount of organic matter entering into the reaction.

Summary. Application of the technic described, considering tissue toxicity as well as germicidal power, indicates that in general the chlorine compounds rate the highest, phenolic compounds next, and organic mercurials rate the lowest as efficient germicides. This corroborates certain previous studies.[4, 8, 9] Tincture preparations in general are not ideal germicides since the solvents are more toxic to phagocytosis than to bacteria.

The toxicity and germicidal end points found in this study indicate that a large number of germicides in general use are too concentrated. Germicides should be utilized in concentrations which are germicidal but non-toxic to tissue. The efficiency range is defined.

Of the natural barriers to infection, *i. e.*, skin and blood, the blood is more sensitive to the destructive action of germicides. Therefore the phagocytosis inhibition technic may be regarded as a valuable aid in the evaluation of germicides for clinical use.