reducing value obtained for the urine. If permutite is used, some time can be saved by adding Lloyd's reagent and the permutite together to the diluted urine. There seems to be little change produced in the results by this slight modification.

The average of a number of determinations gave, per 100 cc. of normal urine, 55.4 mg. "glucose", when untreated Lloyd's reagent and permutite were used, 50.5 mg. when acid treated Lloyd's without permutite was used, 51.5 mg. when acid treated Lloyd's was used with permutite. The error in the recovery of 40 mg. added glucose per 100 cc. was −2.1 mg. when acid treated Lloyd's was used without permutite, and +1.6 mg. when the acid treated reagent was used with permutite.

3927

Effect of Ionization on Antiseptics.

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Results from our studies on antiseptics lead us to believe that the theories which are commonly advanced to explain such action are not satisfactory. To set forth the significance of our results it appears necessary to consider briefly the experimental basis of current theories.

Experiments performed by Krönig and Paul[1] on the antiseptic properties of $HgCl_2$ and the effects of NaCl upon its germicidal properties led these investigators to the general hypothesis that the ionized part of the toxic molecule is the part that penetrates the cell and kills the organism. Their results would lead one to believe that if anything is added to a solution which decreases the amount of ionization of a germicidal molecule, the germicidal value is decreased. They concluded this from the fact that when NaCl is added to solutions of $HgCl_2$ the germicidal value of the solution is decreased. They explained this by assuming first that the Hg ions are the toxic elements, and second, that by the addition of the common Cl ion the ionization of the $HgCl_2$ was lowered. Because salts of mercury ionize very poorly, and because $HgCl_2$ forms complex ions readily with various salts,[2] it would appear that the assumptions

[1] Krönig and Paul, *Z. Physikal. Chem.*, xxi, 419.

[2] Clark, *J. Phys. Chem.*, 1901, v, 289.

made by Krönig and Paul would have to be carefully verified before broad generalizations can be drawn from the effects of NaCl upon $HgCl_2$.

Rideal and Rideal[3] attempt to explain the effects of salts upon germicides by the effects of these salts upon the partition coefficient of the toxic molecule between the bacterial cell and the medium. It is self evident that there are only 2 ways in which this germicidal efficiency of a solution can be increased. One way is to make the molecule more toxic and the other way is to increase the concentration of the molecule within the cell. When germicides are activated by the addition of salts that do not react chemically with the germicides the latter explanation must be the one that applies. When one states that the germicide is activated because of the fact that the partition coefficient is increased, which is equivalent to the statement that the germicide is activated because the concentration of the toxic molecule is increased within the organism, it is merely another way of stating the fact, and is not any explanation of the mechanism. This explanation can be made to fit any case, but does not give an insight into the actual mechanism.

Rideal and Rideal[3] review the literature and show that the phenols and their derivatives are rather powerful germicides whereas their sodium salts are poor germicides. In the form of salts we would expect them to ionize to a large extent, while in the acid form one would expect little ionization to take place.

We have found that the phenols and their derivatives are most active in acid media, while they lose in strength as the pH increases. The following table is a typical example. To obtain this data tests were performed according to Reddish[4] method, using a standard strain of *Staphylococcus aureus* (Insecticide Board strain) as the test organism. The time of medication used was 5 minutes, and the temperature of medication 37.5° C. The numbers given indicate the bacteria surviving as determined by counting the colonies that developed from a standard test sample that was incubated on nutrient agar plates.

TABLE I.

Effect of pH on the germicidal efficiency of phenol and some similar compounds.

Antiseptic material	pH 5	6	7	8	9	10
2.5% Resorcin	180	600	840	1450	1800	3600
.1% B. Naphthol	0	1	11	360	1500	9600
Hexyl Resorcin (1-10,000)	360	600	1900	2700	3600	10000
Phenol (1-1000)	0	0	270	360	470	720

[3] Rideal and Rideal, "Chemical Disinfection and Sterilization," 1921, 182.

[4] Reddish, *Am. J. Pub. Health*, xvii, 320.

These results can be best explained if one assumes that the ionized portion is not antiseptic. In the above solution the germicides and the buffers were mixed just before they were tested. All the buffers used contained the same molecular concentration of salts so that the osmotic pressures of all the germicidal solutions used in the above table were approximately the same. This shows that the effects observed could not be due to differences in osmotic pressure.

This is further substantiated by the next experiment where the germicidal solutions were allowed to age before they were tested. The fact that they lost in germicidal power on ageing cannot be explained by any osmotic effects, or by any change of the organisms that are placed in the germicidal solutions. It would appear that the formation of the salts of these compounds requires time and for that reason the germicidal value of these solutions on the alkaline side would decrease on ageing. The following data show that such is the case:

TABLE II.

Effect of ageing upon the germicidal efficiency of alkaline phenol solutions.

Antiseptic	No buffer	In 2% Na_3PO_4 fresh	In 2% Na_3PO_4 24 hours old
1-80 Phenol	0	800	2400
3% Resorcin	0	0	1000

Similar evidence can be obtained from a study of the antiseptic properties of the basic and acid dyes. Burke and Grieve[5] have shown that the basic dyes are rather weak germicides in acid media but are strong germicides in basic media. These substances will form salts in acid media which should ionize rather well, whereas in basic media they will act like weak bases which ionize poorly. The acid dyes on the other hand behave in the same way as phenol.

If the unionized molecule is the one that penetrates the cell it should be possible to increase the germicidal properties by the addition of common ions and to decrease the antiseptic power by the addition of other ions. Such experiments, however, must be interpreted with extreme caution, as the effect of mass action may interfere as described below. The following data will illustrate this point.

Here all the salts added increased the germicidal efficiency. If one confined his attention to the NaCl he could claim that the common ion depressed the ionization of sodium phenate and thereby increased the germicidal efficiency, and the other salts would appear to give contradictory evidence. One must bear in mind here, however, that a very large amount of the various salts were added in

[5] Burke and Grieve, *Am. J. Med. Sci.*, clxviii, 98.

TABLE III.

Effect of salts on the germicidal efficiency of aromatic compounds.

Antiseptic	Salt added	Surviving bacteria	
2.5% Resorcin	2% NaCl	75	pH 10
.1% B. Naphthal	"	420	"
Hexyl Resorcin (1-10,000)	"	450	"
2.5% Resorcin	No salt	1200	"
.1% B. Naphthal	"	10000	"
Hexyl Resorcin (1-10,000)	"	900	"
Phenol (1-100)	"	450	"
"	2% NaCl	0	"
"	3.8% $CaCl_2$	0	"
"	1.9% NH_4Cl	0	"
"	2.5% KCl	0	"
"	No salt	135	pH 7

comparison to the molar content of sodium phenate present. It appears that the law of mass action would come into play here so that in the case where the KCl was used, the potassium would replace the sodium in the sodium phenate. On this basis one would be justified in calling it a common ion effect in every case of the above table. An identical result would be obtained if one added the same amount of potassium phenate to the solution as there was sodium phenate in the former case and then added the potassium chloride and a small amount of sodium chloride. In this case one would certainly be justified in calling it a common ion effect. The phenal certainly would not know which one of these salts were added first.

To show the effect of added electrolytes that do not furnish a common ion, one would have to use a small amount of the electrolyte in comparison to the amount of the germicide used. In this connection we find that small amounts of KCl added to sodium phenate decrease the germicidal power, whereas NaCl increases it. Tilley and Schaffer[6] found that NaCl added to NaOH and actiseptic sodium salts increased their germicidal activity. A similar effect has also been reported by Levine, Toulouse and Buchanan.[7] They find that NaCl, Na_3PO_4 and Na_2CO_3 will increase the germicidal power of NaOH and they suggest that it is the unionized NaOH which penetrates the cell, and that the addition of common ions depress the ionization. Their data show that for equivalent amounts of sodium added these 3 substances have the same quantitative effect. We have found similar effects of NaCl on Na_3PO_4 and we find further that KCl, NH_4Cl, $CaCl_2$, which do not furnish a common

[6] Tilley and Schaffer, *J. Infect. Dis.*, xxvii, 358.

[7] Levine, Toulouse and Buchanan, *J. Ind. and Eng. Chem.*, xx, 179.

ion decrease the germicidal efficiency. The effect of the KCl, however, is very slight. Since fairly high concentration of Na_3PO_4 has to be used to get any germicidal effect, fairly large amounts of the other electrolytes can be added in this case without obtaining the effect of the mass action as was experienced with the phenol.

Since *Staphylococcus aureus* is very difficult to kill by the phosphates alone, we used for some of these experiments young cultures of *Staphylococcus auranticus,* a less resistant organism. If the *Staphylococcus aureus* were used there would be such a large number of surviving organisms that the experimental error in counting would be too great.

The following data illustrate typical results:

TABLE IV. *Effect of electrolytes on germicides.*

Germicide			Organisms surviving			
	No salt	+NaCl 2%	+KCl 2.55%	+NH_4Cl 1.81%	+$CaCl_2$ 3.78%	
8% Na_3PO_4	540	300	630	10,000	10,000	
*2% Na_3PO_4	760	360	450	2,400	10,000	
	No salt	1% NaCl	5% NaCl	1% KCl	5% KCl	.01%KCl
4% Na Phenate	38	18	26	17	60	100

*For this *Staphylococcus aurenticus* was used.

In view of these results we believe that the evidence is in favor of the hypothesis that the concentration of the unionized portion of an antiseptic is the factor of prime importance in determining germicidal efficiency.

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An Experimental Study of Plasma Protein Regeneration.

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The object of this paper is to study the restoration of the several fractions into which the plasma globulins and albumins may be divided, after severe bleeding of dogs. No general anesthetic was used in the experiments. The dogs were bled from a large vein either in the hind or front leg. Plasma-pharesis was employed to maintain the cell volume and blood viscosity at a high level.