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Use of the Chick Embryo in Evaluating Disinfectants.*

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The recognition of the limited value of the phenol coefficient test in evaluating wound disinfectants has led to a search for methods by which the tissue toxicity as well as the germicidal action of those agents can be determined *in vitro*. While suggested methods^{1,2,3} differ in detail, they all measure the toxicity of disinfectants for tissues and for the test bacteria. If the disinfectant is less toxic for tissue than for the test organisms, it is inferred that the disinfectant is therapeutically active. However, such an interpretation may not be entirely justified, since the problem of wound disinfection is highly complex and the factors that influence the process are diverse. This being the case, efforts should be continued to develop a reliable, easily reproducible and inexpensive method for determining the actual therapeutic value of disinfectants *in vivo*.

In this report, experiments are described indicating the utility of the infected chorio-allantoic membrane of the developing chick embryo in studies on disinfectants. To illustrate the application of this method, results of testing the efficacy of aqueous solutions of phenol, iodine, and cetyl pyridinium chloride are presented.

Using the technic of Goodpasture and Buddingh,⁴ windows were cut in the shells of eggs containing 11-day-old embryos, and a cover slip was placed over the opening of each. On the following day the chorio-allantoic membranes were inoculated with 0.02 cc of a 1:10 dilution (about 1,500,000 organisms) of a 23-25 hr broth culture of *Staphylococcus aureus* (F.D.A. strain 209). Eighteen hours later and on each of the 5 days following, 0.2 cc of the test disinfectant was dropped on the membrane. On the day after

TABLE I.
Therapeutic Effect of Disinfectants on Staphylococcal Infections of the Chorio-allantoic Membrane.

Disinfectant in aqueous solution	Dilutions tested	No. embryos infected	No. surviving infection and treatment	No. survivors showing degree of infection			
				0	+	++	+++
Phenol	1:100	17	14	0	0	0	14
	1:500	16	12	0	0	0	12
	1:1,000	13	8	0	0	0	8
Iodine	1:2,500	19	4	0	0	1	3
	1:5,000	25	16	0	0	0	16
	1:10,000	20	10	0	0	0	10
Cetyl pyridinium chloride	1:2,500	18	7	1	1	3	2
	1:5,000	24	17	0	1	4	3
	1:10,000	13	10	1	1	3	5
	1:20,000	12	10	0	0	1	9
Controls—Saline		30	20	0	0	0	20

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the sixth treatment, the membrane was stroked with a moist cotton swab and with it half of a nutrient agar plate was streaked. The number of bacteria recovered on the petri plate was considered to be an indication of the degree of infection and was recorded as follows: 0 = no colonies; + = 1-10 colonies; ++ = more than 10, but plate not covered; +++ = plate covered with growth.

The results shown in Table I indicate that the infected chorio-allantoic membrane of the developing chick embryo may be used to demonstrate definite and decisive differences in the therapeutic value of disinfectants. These data represent the results of several experiments and since the results of

such tests were similar, one may conclude that the findings are readily reproducible. The method as described makes it possible to determine with accuracy the comparative value of disinfectants under conditions more comparable to infected wounds than can be obtained by any *in vitro* method now available.

Insofar as the disinfectants themselves are concerned, it is evident from the data reported that (1) phenol did not show any demonstrable beneficial action, (2) with the exception of one embryo, iodine had no therapeutic effect, and (3) cetyl pyridinium chloride had a definite curative effect on the experimental infection.

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p-Aminobenzoic Acid and Sulfonamides in Rat Nutrition.

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Nutritional experimentation involving p-aminobenzoic acid¹⁻⁵ and the sulfonamides^{6,7,11,12,13} has disclosed a similarity of action of the two agents. The role of both sulfonamides¹⁰ and p-aminobenzoic acid⁵ in altering the microflora of the intestine suggested the investigation under consideration in this report.

Three series of black rats were placed on diets with and without p-aminobenzoic acid and sulfanilamide.

Basal Diet.	
Vitamin free casein	18
Sucrose	67
Salts	4
Butter fat	9
Cod liver oil	2

Vitamin Supplements per kg of Diet.

	mg
Thiamine hydrochloride	5
Riboflavin	10
Pyridoxine	5
Nicotinic acid	100
Calcium pantothenate	100
Choline chloride	200
Inositol	200

Series 1: 20 black rats, weighing 40 to 50 g, were placed on the above diet containing the 7 B complex vitamins without p-aminobenzoic acid or sulfanilamide. On this diet, as previously reported,⁵ rats develop symptoms which terminate in death in 6 or 7

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