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Tissue Toxicity of the Germicides Iodine and Bromine.*

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Lambert[1,2] as early as 1916, defined the ideal antiseptic as one which kills the infecting agent without causing injury to body cells. In accordance with this definition he evaluated a series of germicides in terms of relative toxicity to *Staphylococcus aureus* and human connective tissue cells respectively. In different later investigations, various other tissues were used for tests of this type. Lampert and Meyer[3] used rabbit spleen, German[4] and Buchsbaum and Bloom[5] used chick tissue culture, while Salle and Lazarus[6] used embryonic chick heart tissue for their experiments. In experiments carried out by Salle and McOmie[7] and Salle, McOmie and Schechmeister,[8] embryonic tissue was used as the test substance. Similar investigations were carried out by Osgood[9] and Herrell and Heilman.[10]

In other methods of determining toxicity for tissue, living chick embryos have been employed as the test object. The living embryo is preferable to tissue culture for this purpose as it presents conditions which approach more closely the complexity of the tissue interrelations of the intact animal. The developing egg presents the additional advantage that it consists of rapidly growing embryonic tissue in a perfect nutritional environment, and furthermore contains large amounts of albuminous material with which the bactericidal agent can come into contact. Moreover, the chick embryo is convenient to handle because of mechanical protection and the ease of repeated access to the embryo which is afforded by the egg shell.

Tests of disinfectants for their potency *in vivo* by chick embryo methods, were carried out by Witlin,[11] Dunham,[12] and Green and Birkeland.[13]

In a third method the toxicity test is carried out in the presence of blood. The use of this test object is indicated because of its known role in infection, and because of the high sensitivity of blood cells to the germicides. Welch and Brewer[14] claim that "application of antiseptics which destroy this function at dilutions which cannot destroy bacteria is a harmful practice". In papers by Nye,[15] Welch and Hunter,[16] and Hirsh and Novak,[17] phagocytes were used as test object of the toxicity assays.

In the following experiments the two last

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TABLE I.
Effect of Halogens on Phagocytosis.

Exp. No.	Halogen	Concentration, %	Polynuclears taking part in phagocytosis, %	No. of bacteria ingested per 100 cells
1	Br_2	1.75	45.7	1.2
	I_2	0.5	13.5	0.27
2	Br_2	1.25	52.0	1.76
	I_2	0.25	25.5	0.57

mentioned methods were used with some modifications.

In earlier tests comparing the disinfectant activities of bromine and iodine, it was observed that bromine *in vivo* (rabbit test) was more active than iodine, whereas the latter was more effective than bromine as a disinfectant *in vitro* (Rideal-Walker test). This suggested that bromine applied on living tissue might be a more efficient antiseptic for use on wounds, cuts and abrasions than iodine.

Toxicity tests on both halogens were undertaken. The method of Hirsh and Novak[17] was employed. In this method the highest dilution which destroys the phagocytic power of leucocytes is determined (toxicity end point) and divided by the highest dilution which kills the test organism (germicidal end point) under the same conditions. Thus, by dividing both end points a toxicity index which expresses the disinfectant power of the tested substance *in vivo* is obtained. A difficulty was encountered in carrying out the test as described by Hirsh and Novak.[17] Even when 7 parts of saturated bromine solution of 3.5% were added to one part of blood marked phagocytosis still occurred. Thus, when the prescribed amount of blood was decreased from 50% to 12.5%, the toxicity end point was still not reached. It is clear, however, that the toxicity index is well under 1, and therefore highly favorable.

In another series of experiments a slight modification of this experimental technique was employed. The germicidal end point was determined as described above, but the harmful effect of the halogens on the leucocytes was evaluated by making blood smears after contact of the blood cells with the halogens for 30 minutes at 37°C in dilution which prevents growth of the test organism. The bacteria were added and a contact time of 10 minutes was allowed, the mixture being shaken thoroughly in the meantime in a water bath of 37°C to assure contact between blood cells and bacteria. After staining the blood smears with Loeffler's methylen blue, 100 polymorphonuclear leucocytes were counted and (1) the number of the cells taking part in phagocytosis and (2) the number of bacteria ingested were determined. By comparing the effect of the 2 halogens, the one more harmful to the leucocytes could be recognized. The results are summarized in Table I.

In a further series of experiments, a modification of Green and Birkeland's[13] method for testing a disinfectant on living embryo was used. Chorio allantois of living embryos was infected with *S. aureus* according to Goodpasture and Buddingh.[18] The amount of bacteria was so chosen that the embryo was killed after 24-48 hours. When disinfectant is added in an appropriate amount, it stops the development of the bacteria, and the embryo can survive the infection. The disinfectant to be tested is used in a concentration which in blank experiments does not have a harmful effect on the embryo. After infecting the chorio allantois with a lethal dose of bacteria, a specific time is allowed to enable the bacteria to begin logarithmic growth. The proper time was determined by agar plate counts. The disinfectant to be tested is added about 3-4 times during 48 hours. By comparing the death rate occurring after treatment with various disinfectants, the relative efficacies were determined.

[18] Goodpasture, E. W., and Buddingh, G. J., *Am. J. Hyg.*, 1935, **21**, 319.

TABLE II.
Antibacterial and Toxic Action of Halogens on Living Chick Embryos.

Bacteria inoculated	Halogen	Conc.,%	No. of eggs under exper.	No. of dead embryos after 48 hr	Death rate, %
$2x10^6$	—	—	23	20	87
$2x10^6$	Br_2	0.2	11	5	45
$2x10^6$	Br_2	0.06	12	6	50
$2x10^6$	I_2	0.08	16	14	87.5
—	I_2	0.08	5	1	20
—	Br_2	0.1	5	0	0
—	Br_2	0.06	5	1	20

In each experiment $2x10^6$ cells of *S. aureus* from 18-24-hours-old broth culture were used. The eggs were placed for 2-3 hours in an incubator at 37°C to allow the bacteria to begin to multiply. The disinfectants were added after 3, 8, and 24 hours. In control experiments saline instead of halogens was added. The embryos were opened after 48 hours. The results obtained with bromine and iodine are given in Table II. These experiments, too, show that bromine in presence of living tissue is a better disinfectant than iodine.

Conclusion. The assertions of Babcock[19] concerning the effectiveness of bromine *in vivo* in treatment of wounds is confirmed by the toxicity tests.

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Mumps Meningo-Encephalitis. Isolation in Chick Embryos of Virus from Spinal Fluid of a Patient.*

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Meningo-encephalitis may occur as a complication of parotitis or as a primary manifestation of infection with the virus of mumps. Whereas in the first instance the time relationships tend to permit a diagnosis of mumps meningo-encephalitis, the diagnosis of the primary encephalitic manifestation has been placed on a firm basis only since the development of a complement-fixation technic by Enders and his co-workers.[1] The demonstration of a rise in complement-fixing antibodies in convalescent serum as compared to the antibody titer in the serum specimen obtained in the first days of the disease may be taken as diagnostic evidence of mumps.[2] By this means, a considerable number of cases of meningo-encephalitis without prior or concomitant parotitis can be diagnosed as due to mumps.[3] Differentiation between antibodies to the soluble and to the virus antigens[4] may be of further diagnostic help.

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