Eighty-five exposures were made, and of these 52% died as the result of either too high a dosage of radiation, of mechanical injuries produced during the operation, or of incomplete closure of the shell so that bacterial infection proved fatal. Forty-one cases remained to undergo partial development. Of these, 5 showed no developmental changes in radiated regions because of too short a period of exposure. Modifications in the other 36 cases were brought about as follows: 43% in the heart and aortic arches, 48% in the eye and forebrain regions, and 9% in tail and limb buds.

While in these experiments mortality is high, due in part to factors other than radiation,[2] nevertheless the method is one well adapted for the localization of injuries during development in such forms as the chick. With the maintenance of a constant dosage, it could be adapted for testing the relative susceptibility of various regions of the embryo at different stages in the developmental period. Certainly, in these experiments, the parts of the nervous system tested, as well as those of the circulatory system, were far more susceptible than such regions as the developing limb buds at a corresponding age.

5676

Relative Toxicity of Antiseptics on Bacteria and Tissues in Cultures.*

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Lambert,[1] Lambert and Meyer,[2] and German[3] believe that an ideal antiseptic is one which kills the bacteria without harming the tissue cells and they have accordingly devised methods of comparing the toxic effects of antiseptics upon bacteria and tissue cells *in vitro*.

Lambert after exposing fragments of connective tissue for several minutes to a saline suspension of *Staphylococcus aureus* transferred them into various concentrations of mercuric chloride, po-

* This investigation was aided by a grant from the Eli Lilly Co.

[1] Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

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

[3] German, W. J., *Arch. Surg.*, 1929, **18**, 1920.

tassium mercuric iodide, potassium cyanide, Dakin's solution, iodine, phenol, tricresol, argyrol, hydrogen peroxide, glycerol, or alcohol. After one hour the fragments were transferred to physiological salt solution where they remained from a few minutes to half an hour until explanted. Although most of the antiseptics killed the tissue cells at concentrations that did not kill the bacteria, a good growth of cells was observed after exposure for one hour to a 1:2,000 iodine solution, a strength sufficient to sterilize the tissue completely in most instances.

Lambert and Meyer placed rabbit spleen fragments in a *Staphylococcus aureus* suspension for one minute, and then for 20 minutes in various concentrations of alcohol, iodine, mercuric chloride, mercurochrome, acriflavin, protargol, albargin, gentian violet, neosalvarsan, or hexylresorcinol. The fragments were then washed twice in salt solution. The best results were obtained with iodine, mercuric chloride, and neosalvarsan. It is possible that in these cultures the cells may have come from the center of the explant where they were partially protected. In a second series they added the antiseptics directly to the culture medium. In these cultures higher dilutions were used, and neosalvarsan alone was less toxic for cells than bacteria, i. e. inhibiting bacteria in dilutions of 1:30,000 to 1:40,000 while cells were only slightly damaged by these dilutions. They concluded that iodine and mercuric chloride are nearer the ideal than some of the newer preparations.

German placed bits of skin of chick embryos (6 to 9 days old) in various dilutions of gentian violet, acriflavin, potassium mercuric iodide, mercurochrome or picric acid for one and 5 minutes, after which they were washed in Locke's solution and mounted as tissue cultures in a medium of plasma and embryonic extract. To determine the lethal points of the bacteria (*Streptococcus hemolyticus, Staphylococcus aureus,* and *Bacterium coli*) he placed bits of muscle and fascia in broth cultures of the bacteria, and then transferred the tissues to the antiseptic solutions. After one and 5 minutes they were planted on agar plates. He believed the "efficiency" of an antiseptic to be directly proportional to its bacteriostatic effect and inversely to its harmful action on the tissues. He found maximal "efficiency indexes" (product of per cent of tissue cultures showing growth by per cent of bacterial cultures showing inhibition) at the following concentrations: gentian violet 1:1600, efficiency of 0.03; acriflavin 1:800, efficiency 0.1914; potassium mercuric iodide 1:50 to 1:1600, efficiency 0.0; mercurochrome 1:400, efficiency 0.0132; and picric acid 1:50, efficiency 0.0975. German

concluded that these results show how far from perfect ("efficiency" of 1.00) the antiseptics are, but added that these are only rough estimates because of the variability of the results. However, it would appear that German's "efficiency indexes" indicate a degree of precision which is in excess of that warranted by his data. Further, the same index may be obtained from a variety of data because it represents the product of 2 variable percentages.

In our study of certain antiseptics we attempted: (a) to develop a method that would render the figures more quantitative and standard than seems to be possible with the methods described above; (b) after some preliminary experiments performed with a similar technique in this laboratory by Dr. R. Herrick, to compare the new antiseptics merthiolate (sodium-ethyl-mercurithiosalicylate) and metaphen (4-nitro-3, 5-biacetoxy-mercuri-2-cresol) with phenol by means of this technique; and (c) to determine whether the tissue culture technique could be simplified by substitution of some antiseptic for at least part of the present aseptic technique.

Experiments were conducted using: (1) cultures of spindle cells cultivated *in vitro* for several months until all of the cells were morphologically indistinguishable from one another ("pure" strain), and (2) mixed cultures derived from the second transplant of periosteum of 12-day embryonic chicks. Although the "pure" cultures gave more precise results, the second-transplant cultures gave almost as consistent results and had the advantage of being more convenient for a standard and easily reproducible test. The culture medium consisted of 2 parts of chicken plasma, one part of embryonic chick extract, and one part of various strengths of the antiseptic dissolved in Tyrode's solution.

A laboratory strain of *Staphylococcus aureus* isolated from an infected human jaw was used as the contaminating organism. A 24 hour colony from an agar plate was inoculated into 3 cc. of beef broth. The 24 hour broth culture was centrifuged and the bacteria taken up in 5 cc. of Tyrode's solution; one part of this suspension being added to 15 parts of embryonic extract. As great differences in the concentration of the bacteria were found to be a cause of variable results, it was found necessary and satisfactory to follow this method.

Five types of cultures were made: (1) antiseptic and bacteria; (2) antiseptic and tissue; (3) antiseptic, bacteria, and tissue; (4) bacteria; (5) tissue. The cultures were made with various concentrations of the antiseptics and then observed for bacterial and

tissue growth after 24 and 48 hours. These time intervals were used since antiseptics are frequently allowed to remain in contact with the tissue cells for extended periods of time. At least 4 cultures were made with each dilution of each of the 5 types of cultures and these generally showed uniform results. Great care in technique is necessary in such experiments because of the small volumes used. The condensed results are shown in the accompanying table.

TABLE I.

Showing Relative Toxicity of Antiseptics on *S. Aureus* and Embryonic Chick Periosteal Cells.

Antiseptic	Dilutions used	Greatest dilution that killed the bacterium used.* $=(A_b)$	Greatest concentration in which cells show approximately normal growth. $=(A_c)$	$\frac{(A_b)}{(A_c)}$
Phenol	1:1, 1:2, 1:3 1:4, 1:5, 1:6 1:7, 1:8, 1:9 1:10 ×100	1:200	1:900	0.2
Iodine	1:2, 1:4, 1:6 1:8, 1:12, 1:15 ×1000	1:2000†	1:4000	0.5
Mercurochrome	1:4, 1:8, 1:16, 1:20, 1:24 1:28, 1:32 ×1000	1:16,000	1:32,000	0.5
Metaphen	1:20, 1:40 1:80, 1:100 1:120, 1:140 1:160, 1:180 ×1000	1:100,000	1:180,000	0.6
Merthiolate	1:20, 1:40 1:80, 1:100 1:120, 1:140 1:160, 1:180 ×1000	1:140,000	1:160,000	0.9

* The viability of the bacteria after exposure to the antiseptics was controlled by inoculating the tissue cultures into beef broth.

† Inhibited bacteria somewhat. A more concentrated solution could not be obtained without using potassium iodide.

Three kinds of results from cultures of antiseptic, bacteria, and tissue (type 3) require separate mention: (a) In concentrations that killed the bacteria in bacterial cultures (type 1), the bacteria in type 3 cultures were killed except immediately surrounding the tissue, where they grew profusely even though the explants were previously bathed for 15 minutes in the corresponding concentration of antiseptic. (b) In type 3 cultures with concentrations of antiseptic allowing bacterial but inhibiting cell growth, the bacteria grew throughout the medium, but most abundantly about the tis-

sue. (c) Again, in type 3 cultures, with concentrations of antiseptic allowing both bacteria and cells to grow, the bacteria soon overran the entire medium and the cells died.

An antiseptic that would kill the bacteria at concentrations that would not harm the cells would have a ratio $[A_b]/[A_c]$ (an index of relative toxicity) of 1.0 or greater. Although merthiolate is the least toxic of the group as tested by us with this method, it is not an "ideal" antiseptic.

The technique here described makes it possible to obtain rather consistent results on the relative toxicity of antiseptics on bacteria and living tissues, especially if some standard antiseptic is run as a check on the method. In stating the toxicity index, the organism used should be named, as is done in the case of the phenol-coefficient, as described by Reddish,[4] and also the tissue. If other types of bacteria are used, it is possible that the method here described or some modification of it will produce an index that will be more useful than the present phenol-coefficient, which does not consider the effects of the antiseptic upon the tissue cells.

5677

Effect of Hypertonic Solutions on Cerebrospinal Fluid Pressure with Special Reference to Secondary Rise and Toxicity.

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The effect of hypertonic solution on the spinal fluid pressure is of vital importance from a clinical standpoint, since Weed and McKibben[1] show that the cerebrospinal fluid volume could be reduced by intravenous injection of hypertonic solution. Cushing and Foley[2] and Foley and Putnam[3] applied this observation to clinical cases of increased intracranial pressure and it was immediately recognized of importance in the treating of head injuries. However, Browder[4] among others pointed out from clinical ob-

[4] Reddish, G. F., "The Newer Knowledge of Bacteriology and Immunology," ed. Jordan and Falk, 1928, Ch. XXII. Chicago.

[1] Weed and McKibben, *Am. J. Physiol.*, 1919, **48**, 512.

[2] Cushing and Foley, PROC. SOC. EXP. BIOL. AND MED., 1920, **17**, 217.

[3] Foley and Putnam, *Am. J. Physiol.*, 1920, **53**, 464.

[4] Browder, Jefferson, *Am. J. Surg.*, 1930, —? 1213.