THE COMPARATIVE RESISTANCE OF BACTERIA AND HUMAN TISSUE CELLS TO CERTAIN COMMON ANTISEPTICS.

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(Received for publication, August 19, 1916.)

A number of chemicals are strongly bactericidal even in weak dilution when tested on bacteria suspended in broth cultures or in salt solution. In the presence of serum stronger solutions are usually necessary, while in order to kill pathogenic microorganisms growing in the tissues, as, for example, in infected wounds, the antiseptic must often be applied in such strength that body cells, as well as bacteria, are injured or destroyed.

An ideal antiseptic is obviously one that will kill the infecting agent without at the same time injuring body cells.

It is not practicable to carry out on infected wounds,—in man, at least,—experiments directed toward the discovery of such a substance. It occurred to the author, however, that in tissue cultures conditions might be made to approximate those in the living organism; for bacteria and tissue cells growing together in vitro may be easily subjected to the same chemical agents and the effect on each be directly observed.

Experiments were therefore undertaken to investigate the comparative resistance of body tissues (wandering cells and connective tissue cells) to various chemicals, including especially a number of those in common use as antiseptics.

Technique.

Human tissues were used throughout the experiments since it was thought that the results would be of more value if clearly applicable
RESISTANCE OF BACTERIA TO ANTISEPTICS

to human beings. We have shown in a former paper that connective tissue and wandering cells can be cultivated in vitro in a modified plasma medium almost as easily as similar tissues of lower animals. Tuberculous and Hodgkin's lymph glands removed at operation and spleens taken out at autopsy a few hours after death were the tissues used. With each of these a migration of large mononuclear cells and connective tissue cells was obtained. The organism used was *Staphylococcus aureus*, chosen first because of the frequent infections caused by it, and, secondly, because it has been shown to occupy a median position among the pathogenic bacteria in its resistance to disinfectants. It is more resistant, for example, than the streptococcus, but less resistant than *Bacillus pyocyaneus*. Staphylococci from two different sources were used; one strain from the throat of an apparently healthy individual, another from a case of furunculosis. The two strains exhibited little difference in their resistance to the chemicals tested.

In a few preliminary experiments the disinfectant or chemical to be tested was added in the desired strength to the plasma medium of the tissue culture. It was found, however, that certain of the substances used, especially iodine and hypochlorites, caused a liquefaction of the fibrin of the clotted plasma so that the cells found no framework upon which to grow and thus the experiment proved nothing as regards the effect of the chemical on cells. Furthermore, the results as regards the destruction of bacteria were also not uniform, probably owing to the imperfect diffusion of the disinfectant through the clotted medium. The limited diffusibility of chemicals through the plasma clot of tissue cultures has been well demonstrated by Rous. It was therefore decided to subject the sterile and bacteria-laden tissues to the action of the disinfectants for a definite period before their incubation in tissue cultures. The tissues were cut into small

2 I am indebted to the surgical staff of the hospital for their cooperation in supplying me with fresh tissue.
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bits of a size suitable for culture preparations (about 0.5 mm. in

diameter). These were washed quickly in one change of isotonic
salt solution to remove the excess of blood and serum, and were
then transferred to a dish containing a 24 hour broth culture of
Staphylococcus aureus diluted five times with isotonic salt solution.
The tissue fragments were allowed to soak for several minutes so as to
become permeated by the bacteria. They were then transferred to
dishes containing varying strengths of the chemicals to be tested. In
these they remained for exactly 1 hour and were then removed to a
dish of isotonic salt solution until put up in culture preparations,—
usually a few minutes to half an hour. A second series was made with
sterile tissues. Two sets of controls were prepared; one of untreated
non-infected tissue and one of infected tissue. The tissue cultures
were prepared as described in a previous paper, using as a culture
medium chick plasma and human serum in the proportions of 1 to 4.
Five preparations were made for each strength of disinfectant used
and for each of the controls. Besides the antiseptics in common use
several other chemicals were tested, and the results are included in
Table I. Many preliminary tests were necessary to approximate
the lethal strengths of the chemicals for bacteria and cells. Since
each experiment was repeated at least once the preparation of about
2,000 cultures was required.

DISCUSSION.

The table shows that in the case of the majority of the chemicals
used (potassium cyanide, phenol, tricresol, hydrogen dioxide, and
alcohol) tissue cells were definitely more easily killed than were
bacteria. With certain other disinfectants the difference was not
so striking. For example, in several experiments with mercuric chlo-
ride it was noted that in a few preparations there was a slight growth
of connective tissue cells after exposure for 1 hour to a dilution of
1:20,000 or 1:40,000, a strength sufficient to kill or markedly in-
hibit the growth of staphylococci under similar conditions. It was
observed, however, that the cells grew out from the centers of the
tissue fragments, not appearing until after 4 to 5 days of incubation.
It was concluded that growth in these cases was due simply to the
### Table I

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<th>Mercapto mercurochloride</th>
<th>Potassium mercuric iodide</th>
<th>Potassium cyanide</th>
<th>Hypochlorites (Oxidan's solution)</th>
<th>Iodine</th>
<th>Phenol</th>
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0 = no growth; + = growth; ± = slight growth.
low penetrating power of mercuric chloride, for cultures in 1:80,000 never showed an active outgrowth of peripheral cells.

Alcohol in the strengths used (5, 10, 20, and 50 per cent) was found to be bactericidal in only the highest strength. On the other hand, it is noteworthy that human cells show no ill effects from exposure to 5 or 10 per cent alcohol for 1 hour. In one series noted there was indeed a better growth of the alcohol-treated tissues than of the controls. Further experiments, however, failed to demonstrate any definite stimulating action on the part of alcohol. The harmful effect of 20 per cent glycerol is probably referable to the partial desiccation of the tissues produced.

Iodine stands out as the one chemical tested to which cells were found to be more resistant than were staphylococci. A good growth of cells was seen after exposure to a 1:2,000 solution of iodine for 1 hour, a strength sufficient to sterilize the tissue completely in most instances.

These experiments afford further experimental evidence of the value of iodine as an antiseptic, and indicate that, at least in weak aqueous solution, it should not, as is often stated, injure or irritate the tissues.

It was observed, however, that iodine has the power of rapidly dissolving fibrin, a property, which, theoretically, should not be conducive to wound healing. A similar action by hypochlorites (Dakin's solution) was also noted. Although the wound-cleansing property of the latter, which evidently depends on this fibrin-dissolving function, is favorably emphasized by Dakin, it would seem that the plastering together of wound surfaces by fibrin, which is thought to facilitate healing, would be prevented by the use on wounds of either iodine or the hypochlorites.

CONCLUSIONS.

The comparative resistance of bacteria and human tissue cells to antiseptics and other chemicals may be easily tested by tissue cultures under conditions which approximate those found in the living body.

A comparative study shows that while human cells (connective tissue and wandering cells) are highly resistant to many antiseptics,
they are in general more easily killed than bacteria (*Staphylococcus aureus*).

Of the antiseptics tested, which include mercuric chloride, iodine, potassium mercuric iodide, phenol, tricresol, hydrogen peroxide, hypochlorites (Dakin's solution), argyrol, and alcohol, the one which approaches most closely the ideal disinfectant is iodine, which kills bacteria in strengths that do not seriously injure connective tissue cells or wandering cells.