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REPORT ON THE DISINFECTING AND ANTISEPTIC  
PROPERTIES OF IZAL.

[FROM THE BACTERIOLOGICAL LABORATORY, OWENS COLLEGE.]

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AMONG the numerous products which have, of late years, been proposed as advantageous substitutes for some of the older disinfectants and antiseptics, izal is undoubtedly one of those deserving very special attention. Observations made by Dr. Klein nearly three years since have shown it to be capable of killing a large number of micro-organisms associated with various diseases, and this it could do even when sufficiently diluted as to cause no detrimental irritation of tissues.

Among the most remarkable features of this compound are its comparative insolubility and non-volatility at the ordinary temperature, properties which it seems difficult to associate with an active disinfectant, but which numerous experiments have proved not to be incompatible in this case. If we add to these the facts that izal can be freely administered internally, used over extensive wounds, or injected under the skin without bad effects, and, finally, that it does not damage surgical instruments, there are sufficient grounds to justify the impression that izal is a substance of considerable scientific and practical interest.

In the course of numerous experiments on well-known disinfectants, I have come to the conclusion that it is difficult to compare the results obtained by various observers owing to the different conditions under which they conduct their observations, and also because the micro-organisms which they use for the purpose of testing the germicidal properties of various substances differ in their degree of resistance according to the way in which they have been cultivated, kept prepared for experiments, etc. Though accepting fully the results obtained by Dr. Klein and other observers, I, at the suggestion of Mr. J. H. Worrall, thought it might be well to test again the germicidal properties of izal. *I naturally avoided conducting my experiments on the same lines as those fully explained in Dr. Klein's report.*

As it was my intention to study carefully the effects which certain disturbing factors might have on the results obtained, I investigated the action of izal on a small number of germs. I selected them so as to get types of the most important forms of pathogenic bacteria which one might have to deal with in practice. These organisms were:—(1) The bacillus tuberculosis (hominis); (2) the bacillus coli communis (which I preferred to the bacillus of typhoid fever for reasons given further on);

(3) the staphylococcus pyogenes aureus ; (4) the bacillus anthracis (in the sporing stage). In the course of the last seven months I have conducted, with the valuable assistance of Dr. F. Coutts, over 115 experiments with these four microbes, paying special attention to the conditions of growth, temperature, dryness, age of germs, etc., etc., which might be expected under ordinary circumstances to influence the resistance of bacteria or the activity of any disinfectant. A careful record of each experiment has been kept, but in order to diminish the bulk of this report a summary only of the results will be given here.

#### I.—ACTION OF IZAL ON THE TUBERCLE BACILLUS.

(a) *Sputum* obtained from a case of advanced phthisis pulmonalis, and found teeming with tubercle bacilli, was allowed to dry on paper for seven days, being kept during that time in a closed capsule in the dark at the temperature of the laboratory (15 to 20°C). Pieces of paper so prepared were then severally steeped, 1° in izal, 2° in izal, one part of which had been diluted with five parts of water, 3° in izal diluted with ten parts of water. In each case the infected paper was allowed to remain in the disinfecting fluid for forty-five minutes, after which it was removed and inserted under the skin of a guinea-pig. In a control experiment, paper smeared with the same quantity of the same sputum, and prepared at the same time and in exactly the same way as the other pieces of paper, was also inserted under the skin of a guinea-pig of the same age and size as the other guinea-pigs.

In all the cases in which the sputum had been treated with izal, fifty-four days after inoculation, no evidence of tuberculosis was found "post mortem," even at the seat of inoculation ; whilst in the control animal tuberculosis was already well marked on the twenty-seventh day, and very advanced on the fifty-fourth.

(b) Similar results were obtained with paper smeared with scrapings of a *tuberculous gland* obtained from a case of recent general tuberculosis, the tubercular matter being allowed to dry as in the previous case.

(c) Fresh tuberculous matter from a cheesy lymphatic gland (tuberculosis of 56 days duration) was made into a thick emulsion with sterilised water. This was mixed with izal, one part of which had been diluted with ten parts of water, and after two minutes the excess of izal was removed with sterilised filter paper. The thick pulp left was allowed to dry for twelve hours, and then a guinea-pig was inoculated subcutaneously with it.

A control guinea-pig was inoculated with exactly the same quantity of a part of the original emulsion of cheesy gland which had not been treated with izal. After 54 days the first animal showed no trace of

tuberculosis at the post-mortem examination. The control animal was already in an advanced state of tuberculosis at the end of three weeks, and the disease was found, post mortem, to be extensive 59 days after inoculation.

*It is evident from the foregoing experiments that izal mixed with ten parts of water will disinfect in 45 minutes dried tuberculous sputum or other tuberculous matter, and that fresh tuberculous products of great virulence when mixed with about an equal quantity of izal of the same strength as above, and allowed to dry at the ordinary temperature for twelve hours, are also completely disinfected.*

I am unable to state how much *shorter time* or *greater dilution* the disinfectant will admit.

In interpreting the results it is necessary to remember that the bacillus tuberculosis, though not known to be a sporing organism, is one which is not easy to kill under ordinary circumstances. This is due to the bacillus being usually embedded in thick mucus or in cheesy products which effectually offer a barrier to some of the best chemical disinfectants (owing to their being usually at the same time capable of causing coagulation of albuminous compounds). The great resistance which the bacillus presents to the effects of drying is another reason why it is so difficult to kill, for as desiccation is not fatal to it, the germ may remain active in the midst of masses too dense to be penetrated by disinfecting solutions of poor penetrating power or incapable of acting for a considerable length of time.

It will be noticed that I have used the inoculation of guinea-pigs to test whether disinfection was complete or not. This I have found by previous and somewhat extensive experience to be the only reliable method in the case of tuberculosis.

## II.—ACTION OF IZAL ON THE BACILLUS COLI COMMUNIS.

The bacillus used in these experiments had been obtained from fatal cases of Asiatic cholera.

Before being used, the microbe had been cultivated for nine days on potato, the growth then scraped off and mixed with sterilised alkaline broth. With the emulsion so obtained, silk threads were impregnated. These threads were allowed to dry for six hours in a sterilised capsule in the dark, the temperature being about 15° to 20°C. After this they were placed severally in izal diluted with 5, 10, 50, 100, and 200 parts of water, and allowed to remain in the mixtures for one minute in the case of the stronger solutions, and for ten minutes in the weaker ones. After this they were transferred to tubes containing alkaline bouillon, some being previously washed in sterilised water, others not. Control

threads not having been exposed to the action of the izal, but having been kept in sterilised water for the same length of time as the other threads had been, were also cultivated in alkaline bouillon.

After twenty-four hours at 36° C. the control tubes showed already typical growth of bacillus coli, but there was no growth in any of the tubes containing threads which had been dipped in izal. These tubes were watched for twenty days, and during the whole of that time no trace of growth could be discovered.

This absence of growth was tested not only by microscopical examination, but also by plate cultivations in nutrient gelatine and agar.

*It is therefore evident that izal diluted with 200 parts of water is a safe germicide for micro-organisms as resistant or less resistant than the bacillus coli communis.*

The bacillus coli communis (or, to be more correct, bacilli having characters associated with that name) is especially suitable for these kinds of experiments; it is constantly found in the intestine. In several enteric diseases, such as typhoid fever, Asiatic and English cholera, dysentery, it multiplies considerably. In many of these cases it is apparently the cause of severe symptoms or complications. It has become evident of late that bacilli of this type may invade other parts of the body than the intestine, and give rise to severe inflammation (peritonitis, pleurisy, meningitis, hepatitis, abscesses, etc.). It is also found outside the body in all media contaminated with faecal matters.

Moreover, the bacillus coli has a considerable power of adaptation, growing well on strongly alkaline, or distinctly acid, on vegetable or on animal media, such as blood serum, milk, urine, faeces, etc., etc. It bears drying better than the bacillus of cholera and resists the action of such disinfectants as carbolic acid, formalin, hydrochloric acid, much better than the typhoid bacillus and the cholera vibrio.

### III.—ACTION OF IZAL ON THE STAPHYLOCOCCUS PYOGENES AUREUS.

The staphylococcus pyogenes aureus was selected as being one of the most resistant cocci. It is widely distributed in external media and is undoubtedly one of the commonest causes of suppuration. It was, therefore, a fair specimen to use for testing the value of izal in the treatment of ordinary wounds.

In this case fresh cultivations of agar were made; after being kept for 48 hours in the incubator at 36°C the tubes were left for another 24 hours at the temperature of the laboratory. The growth was then scraped off, and spread thickly on small pieces of sterilised filter paper. The paper so infected was allowed to dry slowly at the ordinary temperature, in a sterilised capsule, kept in the dark, for three hours.

These pieces of paper were then steeped in izal diluted either with 100 or with 200 parts of water, and left in the mixture for two hours, one hour, or ten minutes.

After these various exposures, the pieces of paper were removed, washed carefully in sterilised water, and dropped into tubes containing alkaline bouillon. In a control experiment the paper was left in sterilised water for the same length of time as the other papers had been left in izal, and then transferred to alkaline bouillon.

From none of the papers treated with izal diluted with 100 parts of water could any growth be obtained. The same was true when izal was diluted with 200 parts of water, except when the exposure was not more than ten minutes in duration.

The bouillon inoculated remained clear for three days (during which it was kept at the temperature of 30°C.), and at the end of that time it was impossible to obtain any evidence of growth by plate cultivations in nutrient gelatine. (A sufficiently large quantity of the bouillon being used in each case to prevent any chance of error.)

In the control experiments well-marked growth was obtained at the end of 24 hours; at the end of 36 hours the bouillon was very turbid. Plate cultivations made with this culture proved that nothing but the staphylococcus pyogenes aureus had grown in the bouillon.

*From this it seems evident that izal diluted with 100 parts of water is a reliable antiseptic for the dressing of surgical wounds, made with the usual antiseptic or aseptic precautions.*

#### IV.—ACTION OF IZAL ON THE BACILLUS ANTHRACIS.

In making experiments with the bacillus anthracis it was not thought necessary to study the action of izal on the non-sporing organism. Spores of great virulence were used. These spores were prepared in the same way as those which I had used in previous experiments, and which I had found to resist ordinary disinfectants in usual dilutions, with the exception of the most powerful chemical agents. Judging by the results obtained with carbolic acid, I did not expect that izal would be capable of killing these spores in a reasonable time, and the results justified my expectations. The most interesting results obtained were those proving the remarkable inhibitory power which even diluted izal had on the growth of the anthrax spores. Thus in alkaline bouillon to which the 100th part of izal had been added it was impossible to get the spores to show any sign of growth, even when kept at a temperature of 36° C. for seven days, no precaution being taken to prevent the volatilisation of izal. The spores, however, were not killed, for after thorough washing in sterilised water and cultivation in fresh bouillon, abundant growth was obtained.

## GENERAL CONCLUSIONS.

Summing up the results of the experiments just recorded I think that *izal* diluted with 100 or even 200 parts of water is a powerful and reliable antiseptic when contact of a sufficient length of time is secured. As an antiseptic it is more powerful than carbolic acid, and, if it be remembered that it causes very little irritation of living tissues, that in moderate doses it is not poisonous, and that, practically speaking, it is not volatile, there can be little doubt as to the immense advantages which *izal* possesses over carbolic acid in many directions.