and I have since then experimented upon ways and means of securing a more stable preparation, and I was efficiently helped in this endeavor by the Abbot Laboratories. We have finally succeeded in reducing the bulk of the dose to the size of a two-ounce bottle and in giving it sufficient stability for practical purposes.

In the course of our experiments we tried a powder form of such composition that the tetiothalein was precipitated upon admixture with water. We found that, under these circumstances, the precipitate was not sufficiently colloidal to secure satisfactory absorbability and good gall-bladder shadow. Nevertheless, since the publication of my experiments on this substance, two powder preparations appeared upon the market, both of which make use of extemporaneous precipitation, which, unfortunately is hardly more than 50 per cent efficient. It may be of interest in this connection to state that we had an opportunity to experiment with mechanically produced colloidal tetiothalein and that this proved practically unabsorbable.

When tetiothalein is injected intravenously or has been successfully administered by mouth and the subject fasts, in course of hours the gall-bladder becomes filled with bile that casts a denser and denser shadow—the density increasing due to absorption of water from the bile and consequent thickening of it. In this manner pictures of the gall-bladder can be obtained by means of the X-rays and not only the size, shape and position of the gall-bladder determined or the presence or absence of gall-stones ascertained, but also its functional quality studied. For, when a meal rich in fat is taken, the shadow disappears normally more or less completely in two hours. Persistence of the shadow, therefore, much beyond this period means abnormality of the function of the gall-bladder.

From all this it will be seen that Roentgenography, the infant among the diagnostic medical procedures, is bidding fair to outstrip all others; and that artificial contrast media have increased its extent of applicability to an almost unbelievable degree.

METHOD FOR TESTING ANTISEPTIC DYES.

BY GEORGE F. REDDISH, PH.D.

In a previous publication, the writer (1) outlined laboratory methods for determining the efficiency of various kinds of antiseptic preparations. The methods described constituted an attempt to simulate to some degree at least the conditions met with in practice. It is, of course, impossible to duplicate practical conditions closely, but these methods do constitute a distinct attempt to approximate practical use as nearly as reasonably possible and consistent with laboratory practice. In devising these tests, the use for which the various antiseptic preparations are intended, the character of the preparation and the method of application were deciding factors in determining the laboratory procedure for each. All that is required of an antiseptic is that it render the pathogenic microörganisms innocuous when it is applied to infected tissue surfaces.

In the paper referred to above (1), page 495, the writer made the following statement:

"There are types of antiseptic preparations and methods of application by means of which infective organisms are merely held in check and not actually killed by the chemical agent used.

The use of wet dressings, irrigations, such as the hypochlorite method of treating war wounds advocated by Carrel and Dakin, the use of bacteriostatic dyes, the proper application of antiseptic ointments and salves, etc., will, when properly applied, be effective in preventing the activity of microörganisms and leave them easy prey to the leucocytes. Such preparations must be tested in a different manner from that used for the ordinary liquid antiseptics."

Referring particularly to antiseptic dye substances, it is evident that the method of laboratory testing would differ very considerably from procedures recommended for antiseptics which remain on infected surfaces but a short time. Since dye substances do remain in contact with the tissues to which they are applied for an indefinite time, they can be considered in the nature of a wet dressing. It is, in fact, a modified wet dressing to this extent that the antiseptic remains fixed in the treated area for an indefinite time and accomplishes a germicidal and bacteriostatic action as well. Since these antiseptic dyes do remain in the site of application for an indefinite time, the laboratory test indicated is one that will simulate this condition as closely as possible.

On page 532 in the above paper, the following statement is made:

"In examining antiseptic dyes, a more complicated situation presents itself. When these dyes are applied to infected surfaces or wounds they penetrate the tissues and are expected to continue their action long after they have been applied. Since it is difficult to be assured of this continued action in all kinds of antiseptic dyes, and since in regulatory work it is not possible to make a complicated, detailed study of each one, for the present it seems consistent to use a test showing actual killing power. For this purpose the filter paper test is applicable."

The method suggested at that time is evidently not indicated for this type of antiseptic preparation and, as a matter of fact, was never used by the writer in the regulation and control of antiseptic dyes. It was suggested against the better judgment of the writer for the simple reason that no applicable method was then at hand, and because no immediate need for control of this type of preparation was felt at that time.

In the meantime, a method has come to the writer's attention which is more nearly applicable for the testing of antiseptic dyes than any so far devised. This procedure was developed in the Pease Laboratories, New York City, by Dr. L. C. Himebaugh (2), but has not as yet been published by him. This method has been used by the writer during the past two years with very satisfactory results. After very careful study of this test, the writer is convinced that this method simulates to a marked degree the conditions under which these dyes exert their antiseptic effect when used in practice.

This method has been tentatively designated by Dr. Himebaugh as the "Cup Plate Method" and is being described here with his permission. While some slight modifications have been introduced by the writer, they are changes which do not alter in any way the general principles of the test nor the results to be obtained. The test is, briefly, as follows:

Staphylococcus aureus of normal resistance (3), (4) is grown at 37° C. in broth (0.5% Liebig's beef extract, 1.0% Armour's peptone, 0.5% sodium chloride in distilled water, adjusted to p_H 6.6 to 6.8) and transferred for three consecutive days. One-tenth of a cc. of a 1–100 dilution of this culture is added to 15 cc. of melted serum-agar (10% blood serum in nutrient agar containing 1.5% agar)

cooled to 45° C., the culture thoroughly mixed in the serum-agar and poured into a Petri dish. A sterile glass stopper or vial 1.5 cm. in diameter is immediately placed in the center of the dish and left until the agar has cooled. As soon as the agar has hardened sufficiently, the glass stopper or vial is removed by twisting slightly and the small cracks and crevices caused by removal of the glass stopper are sealed with two or three drops of sterile agar. Enough of the antiseptic dye solution to almost fill the cup-like depression (0.25 cc.) is placed into the cup and the plate incubated at 37° C. covered with an unglazed clay top. These plates are observed at the end of 24, 48 and 72 hours. If the dye substance is antiseptic and if it penetrates the serum-agar medium in germicidal or bacteriostatic strength there will be a clear zone around the cup. (See Fig. 1.) Subcultures into broth from different parts of the zone are necessary to determine whether the clear zone represents germicidal or bacteriostatic action. However, a clear zone around the cup is all that is necessary to show antiseptic power of the dye substance being tested. If *any* antiseptic solution will penetrate serum-agar of the above composition and after doing so either kill or inhibit the growth of the test organism, *Staphylococcus aureus*, and if the antiseptic when used in practice remains in or on the site of application for a long time, it is reasonable to assume that it will render the infective microörganisms innocuous.

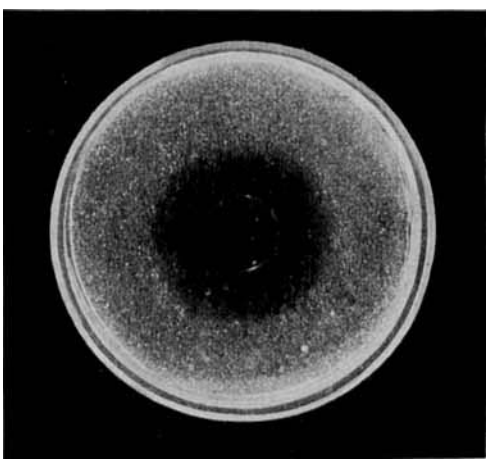

Fig. 1.—This illustrates the penetration and antiseptic action of 2% aqueous mercurochrome in a serum-agar plate containing *Staphylococcus aureus*. This antiseptic dye penetrates the medium for a distance of 1 cm. from the edge of the cup.

The relation between the penetration of a specific antiseptic substance through nutrient agar and the absorption of this same antiseptic through the skin has been well demonstrated by Zeigler (5). He used iodine ointment (U. S. P.) and stainless iodine ointment (N. F.) and tested them for antiseptic action by the method which I described in a previous publication (1). (In these tests plain nutrient agar without serum was used instead of serum-agar.) He then studied the absorption power of both these ointments through the skin by the method described by Witzel and Sollman (6). Iodine ointment (U. S. P.) gives a wide clear zone by the agar plate test and the iodine from this ointment readily penetrates into and through the skin. On the other hand, stainless iodine ointment (N. F.) does not give a clear zone on the agar plate and when tested for absorption the iodine is *not* absorbed through the skin. Because of this very definite correlation between penetration of iodine through nutrient agar and absorption through the skin, it is consistent to interpret this laboratory test in terms of antiseptic efficiency when such preparations are used in practice. The same interpretation is made of the agar cup-plate test. When the antiseptic dye penetrates the agar medium (in this case serum-agar) and forms a clear zone around the cup, it is reasonable to expect that

it will also penetrate the skin and body tissues and exert its antiseptic action there in a similar manner. Of course the absorption of different chemical substances through the skin will vary considerably, but antiseptic dyes are well known for their ability to penetrate the skin and body tissues. Therefore, the penetration of antiseptic dyes through serum-agar to give a clear zone around the cup is a safe index of the antiseptic action that can be expected of these dyes when used in practice.

SUMMARY.

A method for testing antiseptic dye solutions is described. This method is the one recommended by Himebaugh of the Pease Laboratories, New York City, in 1927, for determining the penetrating power and antiseptic action of antiseptic solutions in general. The method is eminently suited for testing the antiseptic action of dye substances, and is the only applicable test which has so far come to the writer's attention. It is recommended that this method be used as a standard procedure for testing antiseptic dye substances.

BIBLIOGRAPHY.

(1) George F. Reddish, "Methods of Testing Antiseptics," *Drug Markets*, 20 (1927), 495.

(2) L. C. Himebaugh. Read at the annual meeting of the Society of American Bacteriologists, Rochester, N. Y., Dec. 29, 1927.

(3) George F. Reddish, "The Resistance to Phenol of Staphylococcus aureus," *Amer. Jour. Pub. Health*, 15 (1925), 534–538.

(4) George F. Reddish, "Examination of Disinfectants," *Am. J. Pub. Health*, 17 (1927), 320–329.

(5) W. H. Zeigler, "A Study of the Absorption and Antiseptic Properties of Several Types of Iodine Ointments," JOUR. A. PH. A., 17 (1928), 648–650.

(6) Witzel and Sollman, "Iodine Absorption from the Human Skin," *Journal Pharm. and Exper. Therap.*, 15, No. 2 (Apr. 1920), 169.

FRENCH TECHNICAL SCHOOL OF APPRENTICESHIP FOR THE DRUG, PERFUMERY AND RAW PRODUCTS TRADE.

The lectures on crude organized by the Wholesale Druggists' Association, of Paris, for the benefit of their employees are to be transformed to a "Technical School of Apprenticeship for the Drug, Perfumery and Raw Products Trade." This action has been taken in view of the new apprenticeship tax, of which the proceeds are to be devoted to technical education. The Association has demanded that their trade be placed on the same footing (as regards the tax) as the perfumers, and it has been arranged that 70 per cent reduction shall be accorded for professional education and formation of skilled and half-skilled labor, 20 per cent for formation of staffs and laboratories and 10 per cent for household instruction. The Minister of Public Instruction allocates an annual sum of 2500 fr. to the school.—Through *Chemist and Druggist*, of January 26th.

CHEMICAL NATURE OF PEPSIN.

Pepsin is difficult to investigate chemically because its aqueous solutions are rapidly inactivated by dilution and agitation, and partially also by aeration. The addition of alcohol or acetone in presence of mineral acids converts pepsin into an insoluble compound from which the enzyme cannot be regenerated. In presence of picric acid or its salts, pepsin becomes more stable. It can be precipitated quantitatively from concentrated solutions, but it is uncertain whether the precipitate is a true compound or merely an adsorption combination. The fact that some precipitates have greater stability and that some reagents render pepsin totally inactive is held to be evidence that true compounds are formed. The solubility of the sodium and barium compounds of pepsin may indicate that salts are formed with the acid groups in the pepsin molecule—the digestive milk coagulating power. There is also evidence that pepsin consists of an inactive fraction and a kinase.—Through *Chem. Abstr.*