tains visible parts homologous to a leg consisting of coxa, femur and tibia.

The following stages of transformation are typical, omitting more or less abnormal side lines. (1) The wing is spread at right angles, small irregularities of venation appear; the costal cell changes. (2) The spread wing shortens, part of the veins and of the wing at the posterior end are missing. The costa adjacent to the costal cell becomes thicker and its segmentation into three parts visible in the normal wing is accentuated. The costa thickens and extra bristles appear in addition to the normal two rows. (4) The wing spread is reduced to two lobes, frequently inflated or in the shape of two chitinized knobs with bristles; only the squama (alula) remains more or less as a separate posterior structure, sometimes assuming the shape of a palpus. At this stage the costa and the neighboring cell become separated from the rest of the wing and transform into a typical leg without tarsus, sometimes properly bent between femur and tibia. There is a coxa at the basis, tibial spurs at the end of the tibia and the four rows of bristles on each face typical for a leg. There are many small variations and pathological formations, but this main line of transformation clearly stands out. Sometimes the incomplete transformation shows in an anterio-posterior sequence; (1) the more or less developed leg, (2) two knobs of different size and form and with bristles, (3) a palpus-like structure derived from the squama. The best description of this condition would be to call it a quadripartite parapod with one leg and palpus and two lobes in between. It ought to be added that the halteres are always normal, but that in many individuals femur or tibia in one or two legs show abnormalities.

These facts demand a discussion from the standpoint of comparative morphology. I can not see any way out of the following conclusions (which will be analyzed in detail and with discussion of former views in a forthcoming paper) except by claiming that what looks like a three-jointed leg, in the best cases of about two thirds size of the normal leg without tarsus, is no real leg: The insect wing is a dorsal homolog to a ventral leg, both primarily consisting of coxa, femur and tibia exactly as in Snodgrass's picture of an ancestral leg. As functional dorsal legs are an impossibility the origin of the wings must go back to the dorsal parapodium (notopod) of polychetes. The quadripartite condition of the wing must be derived from a similar condition in a parapod. It is possible that thus the squama parallels an exopodit, while the basal part of the costa remains the leg or endopodit. The general phylogeny of arthropods will have to be revised if our interpretation of the new facts is correct.

RICHARD B. GOLDSCHMIDT
UNIVERSITY OF CALIFORNIA,
BERKELEY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SUGGESTED STERILITY TEST FOR PENICILLIN

IN 1943 the authors[1] reported the inactivation of penicillin by cysteine.[2] Lawrence,[3] in 1943, described a sterility test for penicillin which involved the use of an enzyme preparation, Clarase, for the inactivation of the antibiotic. In a later communication[4] he stated that this activity of Clarase was not associated with its diastatic properties, but was due to water-soluble filterable substances of bacterial origin which were present in the enzyme preparation. Stanley[5] reported that only certain samples of Clarase were effective penicillin-inactivators and emphasized the fact that each lot of the material should be tested and standardized in this respect before being employed in the sterility test. Recently, Cavallito and Bailey[6] published a note dealing with the inactivation of penicillin and other antibiotics by cysteine and related compounds. Harper,[7] Ungar[8] and Liebmann, McQuarrie and Perlstein[9] have described penicillinase preparations from different bacteria, and the last-mentioned workers have stressed the need for a penicillin-inactivator which can be sterilized and standardized easily.

In our laboratories, the following compounds have been tested for their ability to interfere with the antibiotic activity of penicillin: cysteine, cystine, glutathione, methionine, taurine, thiosalicylic acid, thiourea, sodium thioglycollate and dithiodiglycol. These compounds were dissolved in NaOH–KH_2PO_4 buffer solution or water, adjusted to a pH of from 7.0 to 7.5 and sterilized by filtration through Swinney or Seitz filters. A concentration of 2 per cent. was employed in each case where the compound was

[1] Report to the Johnson Research Foundation, 1943.
[2] This work was carried out at the Laboratories of General Bacteriology, Yale University, and was aided by a grant from the Johnson Research Foundation of New Brunswick, N. J.
[3] C. A. Lawrence, SCIENCE, 98: 413, 1943.
[4] *Idem*, SCIENCE, 99: 15, 1944.
[5] A. R. Stanley, SCIENCE, 99: 59, 1944.
[6] C. J. Cavallito and J. H. Bailey, SCIENCE, 100: 390, 1944.
[7] G J Harper, *Lancet*, II: 569, 1943.
[8] J. Ungar, *Nature*, 154: 236, 1944.
[9] A. J. Liebmann, E. B. McQuarrie and D. Perlstein, SCIENCE, 100: 527, 1944.

soluble to that extent; otherwise, saturated solutions were used. In the case of cystine, a 2 per cent. suspension of crystals was sterilized in the autoclave rather than by filtration. Each solution (or suspension) was tested by mixing 2.0 ml with 1.0 ml of sterile penicillin solution containing 200 Oxford units, incubating for one hour at room temperature and then transferring the mixture to 20 ml of thioglycollate broth. The thioglycollate broth tubes were inoculated with *Staphylococcus aureus* and incubated at 37° C. Experiments were also conducted in which mixtures of these compounds and penicillin were assayed for antibacterial activity after different periods of incubation. The fact that cysteine was the only reagent found to exert an appreciable effect upon penicillin activity indicates a high degree of specificity in the reaction between these two substances.

The possible usefulness of such a reaction in testing the sterility of penicillin samples became apparent and a method for this purpose has been developed. The technique is essentially the same as the sterility test for penicillin employing Clarase, except that a 2 per cent. solution of cysteine-HCl is substituted for the 4 per cent. solution of Clarase and a small amount of sterile peptone is added to the mixture for its protective effect on some organisms. In practice, a 2 per cent. solution of cysteine-HCl is prepared in NaOH–KH_2PO_4 buffer at pH 7.5. The reaction is readjusted to this pH level by the addition of the necessary amount of 10 per cent. NaOH. This cysteine solution is placed in 15 mm Pyrex test tubes in 10 to 15 ml amounts and sterilized by autoclaving for 10 minutes at 15 pounds of additional steam pressure. A 5 per cent. aqueous solution of peptone is also sterilized in the autoclave. The penicillin sample is dissolved in sterile water so that 1 ml contains 5,000 Oxford units. A 2.0 ml volume of the cooled cysteine solution is mixed with 1 ml of the penicillin solution in a sterile tube and 0.15 ml of the sterile 5 per cent. peptone is added. This mixture is allowed to remain for 30 minutes at room temperature and then transferred aseptically to 20 ml of fluid thioglycollate medium (thioglycollate medium made according to J. H. Brewer). The latter is incubated at 37° C for 7 days.

This method has been tested for its efficacy by inoculating the reaction mixture or the penicillin with organisms which are capable of growing in the thioglycollate medium. In such experiments we have succeeded in obtaining positive cultures when as few as 35 *Staphylococcus aureus* cells, 15 *Bacillus subtilis* spores or 15 to 25 spores of *Clostridium sporogenes* were added. The numbers of viable cells in the inocula were determined by plate or deep agar tube counts.

The effect of the pH of the cysteine solution on penicillin inactivation and on the survival of test organisms was studied between pH 6.5 and 8.0. In experiments with H-ion concentrations between pH 7.0 and 8.0, growth of test organisms appeared somewhat earlier than in those in which the reaction had been adjusted to between pH 6.0 and 7.0.

We have found that sterile cysteine solutions retained their activity with respect to the above test for more than one week when stored in Pyrex tubes at room temperature. However, it is considered a good policy to use solutions not over three or four days old. The capacity of cysteine solutions to inactivate penicillin was also studied by the above procedure, and these results indicated that 2.0 ml of 2 per cent. cysteine was sufficient to neutralize the antibacterial activity of at least 15,000 Oxford units of penicillin in 30 minutes.

This test has been applied, together with the Clarase sterility test, to at least 10 different samples of penicillin with comparable results in all cases. These samples were presumably mixtures of the F and G types of penicillin. In addition to these, a sample containing a large percentage of penicillin X has been tested and found to be inactivated.

Attempts to simplify the above sterility test by incorporating the cysteine-HCl directly in different test media (both with and without sodium thioglycollate) have not been successful.

SUMMARY

The proposed sterility test for penicillin, which employs cysteine (in the form of its hydrochloride) rather than Clarase, has proven to be satisfactory in our laboratories. The use of cysteine is advantageous because it is a compound of known structure and is readily available at relatively small cost. Suitable samples of Clarase or bacterial penicillinases are not as reproducible and the latter require much time and effort in their preparation. Furthermore, cysteine solutions do not require filtration but may be sterilized by heat in the autoclave.

ADDENDUM

After the above article had been accepted for publication, a paper which dealt with the same subject appeared (R. J. Hickey, SCIENCE, 101, pp. 232–234, March 2, 1945). Our findings are in complete agreement with his except for the fact that we were unable to obtain growth of *Staphylococcus aureus* from penicillin solutions when the cysteine-HCl was incorporated in the thioglycollate broth. Re-examination of this point has indicated that, while the small inocula which we had customarily employed failed to develop in the cysteine-thioglycollate medium, larger inocula

resulted in positive cultures. The inoculum used by Hickey is not described. We feel that the apparent discrepancy between his results and ours may be due to the numbers of organisms employed.

ROBERT D. MUIR
GEORGE VALLEY

PENICILLIN DIVISION,
CHEPLIN LABORATORIES, INC.,
SYRACUSE, N. Y.

A SIMPLE DEVICE FOR THE ADDITION OR REMOVAL OF SOLUTIONS OR GASES TO A CLOSED SYSTEM

THE aseptic removal of samples of gas or media from a closed bacteriological system has been simply and effectively accomplished using sleeve-type, soft rubber stoppers, such as those used to cover serum bottles. This device, described below, also provides for the aseptic introduction of nutrients, buffers or other materials without opening the system or otherwise interrupting the experiment.

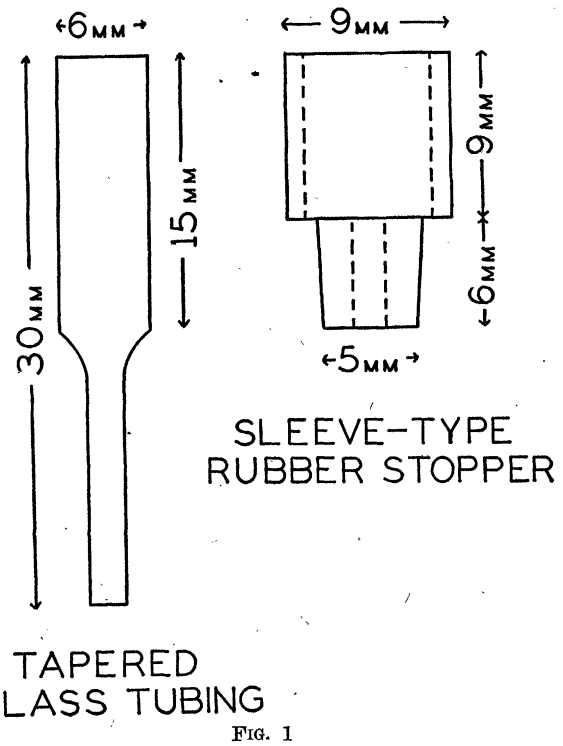

FIG. 1

A sleeve-type, soft rubber stopper measuring 5 mm at the base (see diagram) is inserted in a glass tubing of 5 mm inside diameter or slightly smaller. This tubing is pressed into a hole in the rubber stopper or closure for the bacteriological system. It is usually advisable to taper the tube for a better fit (see diagram). The media or gas may be removed by inserting a hypodermic needle (20 gauge, 1½ inch length), attached to a syringe or sampling device, through the sleeve-type rubber stopper. Several precautions are necessary. The stopper itself should be selected with care to insure sufficient thickness to the top wall so as to cut the exchange of gases to a minimum and in order to provide for adequate re-sealing if frequent punctures are necessary. The glass tube and sleeve-type stopper should be fitted with care to avoid leakage at this joint. The glass tubing should be cut off flush with the bottom of the closure stopper since complete removal of all gas bubbles would become impossible.

The author has found such a sampling device of indispensable value in the study of gas-producing organisms of certain marine bottom deposits. The gases formed in the culture system are collected directly in the Orsat gas analyzer with a hypodermic needle attached by a short rubber tubing to the inlet stopcock of the apparatus. Hydrogen-ion concentration or oxidation-reduction intensity changes can be followed at any desired interval by use of a sterile Luer-type syringe and hypodermic needle for the withdrawal of suitable aliquots. In a similar manner, samples are collected for other chemical analyses, for population estimations or for the transfer of cultures.

JOSEPHINE BECKWITH SENN

SCRIPPS INSTITUTION OF OCEANOGRAPHY
OF THE UNIVERSITY OF CALIFORNIA,
LA JOLLA
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RAPID STAINING METHOD FOR RELAPSING FEVER SPIROCHETES

IN the course of certain experiments the modification of the Field stain described by Medalia, Kahaner and Singer[1] was used. These authors have reported its use in malaria smears, differential blood counts and smears of other cell-containing body fluids. We have found the stain to produce results in spirochete infected blood comparable to those obtained by the conventional methods employing the Wright or Giemsa stains.

To stain the spirochetes, thick films prepared and dried in the usual manner are laked in distilled water or 2 per cent. acetic acid and stained in essentially the same manner as described by Medalia *et al.* The spirochetes stain a deep purple. Actual staining time is 10 to 15 seconds (compared to the 30 minutes required by the more popular Giemsa technique). This rapid method has been used successfully by the author in both animal and human infections of relapsing fever.

PFC. CHARLES L. WISSEMAN, JR.

ARMY SPECIALIZED TRAINING UNIT NO. 1809,
SOUTHWESTERN MEDICAL COLLEGE,
DALLAS, TEXAS

[1] Leon S. Medalia, Jack R. Kahaner and Arnold J. Singer, *Amer. Jour. Clin. Path., Tech. Sect.*, 8(4): 68–70, 1944.