several frames and also to superimpose the track of the eye movements upon the picture which the subject may be asked to observe during the experiment.

The registration and analysis of eye movements were undertaken as part of the study of the physiological modifications occurring in two forms of attention or adaptation investigated by Burrow and his co-workers. The investigation was undertaken especially with regard to disturbances in attention, as we found that a primary deflection in the process of attention was an invariable accompaniment of disorders of behavior, both individual and social. We had previously found specific alterations of the respiratory function (*1*) and of the electroencephalic potentials (*2, 3*) to be concomitant with these modifications in attention and behavior. These instrumentally measurable and verifiable aspects confirm the distinction made between the two types of attention: (a) *ditention,* as seen in "normal" as well as in neurotic behavior, and (b) *cotention,* which represents the organism's primary phylobiology or its healthy basis of orientation.

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Inactivation of Penicillin by Zinc Salts

Hans Eisner
Instituto Andrómaco, Montevideo, Uruguay
Bernardo Porzecanski
Instituto de Higiene, Facultad de Medicina de Montevideo

The Oxford investigators and others have reported that penicillin is inactivated by contact with various heavy metals.

In connection with experiments to determine quantitatively the influence of certain pure metals and their salts, we found that the time needed to inactivate the penicillin is quite different for the various metals and salts. The inactivating process depends on different factors and generally takes several days to complete. Zinc salts (*i.e.* sulfate, acetate, chloride, oxide), however, showed an exceptional behavior in our experiments and inactivated penicillin in a short time. That this particular action of the zinc salts, even in such a low concentration as that used under the conditions of our experiments (1/300–1/60 M) has no perceptible bacteriostatic effects calls for special attention. The inactivation with a well-defined, inorganic, easily sterilizable, and stable substance, such as zinc salts, seems to us to be preferable in some cases to procedures with enzymes or other organic compounds; at least, this method may be of interest as a complement or control of another base. The zinc inactivation method is quite useful to us in determining the effects on penicillin of metals and their salts, particularly because in the presence of these substances the use of enzymes sometimes offers serious difficulties. This preliminary report of the "Zn-inactivation method" may be valuable in cases where usual inactivators have thus far been employed.

Experimental Technique

Penicillin: We employed commercial penicillin-sodium of several manufacturers.

Test organism: The H strain of the Oxford investigators or another coagulase positive strain of *Staphylococcus aureus* sensitive to penicillin was used.

Inoculum: Culture media were inoculated with 3 drops (3/50 ml.) of a 12- to 18-hour broth culture.

Culture media: For the serial broth dilution method we used infusion broth (meat infusion and peptone), adjusted to a reaction of about pH 6.5±, distributed as a routine in small test tubes, 2 cc. for each tube. For the Oxford cup method we have employed ordinary nutrient agar (the same infusion broth solidified with 2½ per cent agar).

Zinc sulfate: Sterile solutions in water of $ZnSO_4 \cdot 7H_2O$, 5, 10, 25, and 50 per cent, were employed.

Procedures: The inactivation of penicillin by zinc sulfate can be studied by various simple and satisfactory routine procedures.

In the *Oxford cup method,* to each cubic centimeter of nutrient agar, melted and seeded with *Staph. aureus,* 1/50 ml. of one of the zinc sulfate solutions is added according to the desired concentration. We use for this purpose a capillary pipette, standardized to 1 drop = 1.50/ml. The agar is poured into plates, and the glass cups are placed on the agar and filled with the penicillin solution to be studied. Incubation is at 37°. The inactivation of penicillin is recognized by bacterial growth throughout the whole plate.

An *inverted* cup assay is as follows: To nutrient agar melted and seeded with *Staph. aureus,* as already described, the desired amount of penicillin solution is added. The mixture is poured into plates; glass cups are placed in the agar and filled with the corresponding zinc sulfate solution. Penicillin inactivation is judged by observing a zone of *Staph. aureus* growing around each cup. On the rest of the plate there is no bacterial growth.

The broth dilution method consists of adding 1/50 ml./cc. of one of the zinc sulfate solutions to a single tube with penicillin solution in normal saline or pure water, alone or with body fluids. Incubation is at 37°. After adequate time (12–24 hours or more), serial

dilutions are made out of this tube, and *Staph. aureus* added to each of these. Incubation is at 37°.

A control tube of penicillin solution without zinc sulfate, stored at 37°, during the same time, is used for comparative measuring of the penicillin potency in serial dilution.

Another assay is as follows: Each tube of a range of graduated penicillin dilution is inoculated with *Staph. aureus,* and zinc sulfate solution is added to the tubes immediately (or after a longer or a shorter time). After some hours (4 or more) the tubes are examined for evidence of growth (reading turbidity).

Discussion

In the inactivation of penicillin by zinc salts are involved factors about which we will report in detail later. Thus far our results seem to indicate that penicillin inactivation by zinc sulfate is closely similar in its course to the penicillinase inactivation process. In both cases, curves which derive from the rate of inactivation significantly differ from the straight line.

In the zinc inactivation method, pH and zinc concentration are of primordial importance. The inactivation rate increases with the concentration of the zinc sulfate employed. When strong zinc sulfate solutions are used, their possible antibacterial effect must be considered. Concentrations of 1–5 mg./cc. liquid medium, as used by us, have no bacteriostatic action in our experiments. Lower concentrations have slow or no penicillin inactivation effect at all. Here it will be of interest to mention that very low quantities of zinc sulfate (1–3 mg./liter) had even been recommended for the stimulation of the penicillin production.

A pH between 6 and 6.8 is preferable for the inactivation process and also appropriate for the staphylococcus growth. The broth used by us allowed concentrations of 1–5 mg. of zinc sulfate per cubic centimeter without need for pH correction. It should be noted that in using the zinc concentrations recommended by us, especially the higher ones, zinc hydroxide may be precipitated out. This does not affect the inactivation process; neither does the zinc hydroxide seem to precipitate or adsorb any appreciable quantity of penicillin. On account of this we use in dilution tests the clear supernatant fluid, even though the same results may be obtained using the fluid with the suspended sediment. Under the conditions of our experiments, 1–5 mg. zinc sulfate inactivate in a few hours (12–24 hours) concentrations of the order of 100 units of penicillin. We have obtained the same results whether serum (human), blood (rabbit) or agar were in the medium. This fact, we believe, makes the zinc inactivation method suitable for culturing blood and other body fluids containing penicillin, for the sterility test of penicillin powder, and, perhaps also, a valuable aid in penicillin assay in mixtures of penicillin with other germicides.

Up to the present, as a result of these studies, we cannot say anything substantial concerning the underlying mechanism of the penicillin inactivation. A purely chemical reaction is highly improbable, considering the amounts of zinc salt in relation to penicillin used.

A Note on Staining Plasmodia

A. W. Ratcliffe

Department of Pathology, St. Mary's Hospital, Inc., and *Department of Biology, Evansville College Evansville, Indiana*

Review of the historical background of malaria has suggested that our conception of plasmodial staining reactions may be incomplete (*3*). The following observations have heightened this impression:

(1) Blood infected with *P. vivax*[1] (produced clinical and typically smear-positive malaria upon therapeutic inoculation), smeared and stained with Wright's stain, was examined by various technicians and by consultant malariologist without recognition of the plasmodium.

(2) Similar blood showed numerous intraerythrocytic organisms, some of which were actively motile, but freshly made and stained smears were searched by a college class in parasitology without demonstration of a single typical parasite.

(3) Smears of blood containing numerous parasites, *P. vivax* (which stained readily when first smeared in Texas), could not, after transportation to Indiana, be stained to show organisms of diagnostic acceptability.

It appeared in this light that solution of the problem of staining these organisms was essential and prerequisite to progress in morphology and also might be helpful in the ancient and recurring problem of smear-negative patients progressing to necropsy demonstration of plasmodia.

Representative of the current concept is the following: "The dilution of the stain and time required for fixing and staining may be varied considerably without changing the quality of the result very much. The pH of the buffer, age of smear, and brand of stain used are important factors which determine the quality of the result" (*2*). Recommendations as to pH range from 7.2 to 6.4. It is indicated that older smears require a lower pH and may require somewhat longer staining time, and that alkaline buffer produces a dark smear while acid buffer produces a red smear.

[1] Indiana Central State Hospital strain, received from Indianapolis as fluid blood.