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

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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.

In recent years the only specific suggestion for staining old blood films seems to be the method of Daniels (1907) which, as outlined by Craig (*1*), calls for a preliminary acid-alcohol bath followed by washing and staining in the usual manner. Repeated trial of these methods did not give the desired result. While the gross appearance of the stained smear is more red with an acid buffer in the range mentioned and darker blue with an alkaline buffer, the red was chiefly that of eosin and the blue largely the result of precipitated stain. Our problem was particularly one of failure to obtain (red) staining of the chromatin. Microscopically, despite the red cast with acid buffers, it was the blue which seemed to stain the nuclei of the leucocytes, rather than the azure components of the stain.

This recalled that hematoxylin, also a nuclear stain, is retarded by acid and precipitated by alkali. Moreover, its staining action is hastened by preliminary rinse of tissue sections in the alkaline bluing bath. Trial of Wright's stain showed clear blue in acid solution but purple with a slight precipitate in alkaline solution. Methyl green was blue in acid but purple in alkaline medium. Azure II showed little change. Azure I and Azure B showed darker blue in the acid solution.

On this basis it seemed that azure-staining of the chromatin might be improved and undue precipitation avoided by using alkali as a preliminary bath rather than as a medium for the stain.

Actual use has verified this principle in several recent clinical cases where typical plasmodia could not be found by routine strain but were present, numerous and well stained after the procedure described.

Sodium carbonate and ammonium hydroxide have both been satisfactory. The concentration is best adjusted by pH determination. Fresh smears will not tolerate strong alkali, and brief exposure to pH 8–9 after fixation may suffice. Older smears are not damaged by considerably stronger alkali and seem to require it for comparable effect. Because of this variation it is not yet possible to be specific as to concentration or time. At present the strength is adjusted to give partial, but not complete, hemolysis. The slide is washed and then stained. For some, the usual time suffices, while for other bloods, prolonged staining is a necessary and helpful factor. This alone gives better results than the routine method, but the use of both the alkali and prolonged staining is distinctly preferable. The nuclei of leucocytes should be distinctly overstained, dark reddish-purple. The staining time is perhaps best controlled by this.

By using several hours in the alkaline bath and 24 hours in Giemsa stain, or a comparable solution of Wright's stain, the parasites brought from Texas in March 1944 have finally been stained to present the desired classical picture.

This seems too useful a tool to withhold, although it needs further development. The possibility of materially hastening the result by adding some penetrant (such as Tergitol-7) is being investigated.

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Letters to the Editor

The Fluorescence of Radium Burns

The fluorescence of scar tissue has been scantly mentioned in past writings in this field. However, it is now widely assumed that cicatrices due to mechanical, electrical, and chemical trauma show little by way of a distinctive response to filtered, long-wave-length, ultraviolet light. Usually tissue damaged by these agents appears lavender or purple to the unaided eye. This excludes the possibility that such scar tissue may contain traces of porphyrins or related substances which would produce a bright red fluorescence, a subject yet open to investigation.

Moreover, it has been assumed that cicatrices due to the effects of X-ray and radium radiations are characterized by a brilliant pearly-white fluorescence under ultraviolet light.

The writer has personally observed numerous instances in which X-ray damage to tissue on various parts of the body fluoresced the pearly-white color. However, it has recently been possible for him to compare the fluorescences of X-ray cicatrices with those caused by radium. In two cases, both adult male and female, tissue damaged in radium therapy not less than 10 years previously exhibited a brilliant light-blue fluorescence under filtered, long-wave-length, *i.e.* 3,650 A., ultraviolet light. In the case of the female, radium irradiation had been employed 15 years previously to remove a small growth on the cheek. The cicatrix was circular, showing a bright-blue fluorescent periphery and a nonresponsive center. The female subject was 25 years of age. In a 55-year-old male, radium had been used 12 years prior for treatment of a growth covering the lower, external