$HgCl_2$-plasma does not manifest Ac-globulin activity, and the antithrombin titer is extremely low. The prothrombin concentration of the $HgCl_2$-plasma when the latter is stored at −20°C to −60°C for 4 months remains unaltered.

In all prothrombin determinations of the $HgCl_2$-plasma, the modified two-stage method of assay was used(11). However, because no Ac-globulin is present in the $HgCl_2$-plasma, it is necessary to add a larger amount of beef serum (Ac-globulin) to the incubation mixture than is normally used. We have found that a 1-400 total dilution of beef serum insures complete conversion, but even with this concentration, the conversion rate is slower than it is in normal plasma.

The method of assay of Ac-globulin, as well as studies on the kinetics of the conversion phase of the clotting system, will be presented in detail in a subsequent paper. A modification of the 2-stage procedure(8) has been devised. It is sufficient to present here only the tenets on which the quantitative estimation is based. The amount of Ac-globulin in any given plasma is manifested as a function of 3 variables: (1) the time when prothrombin conversion begins, (2) the rate of prothrombin conversion, and (3) the yield of thrombin. Therefore a quantitative measure of Ac-globulin can be represented by an area which is circumscribed by the activation curve (clotting time plotted against incubation time) and by straight lines through a constant, arbitrarily selected point on each axis. This area can be determined by the square counting method, one of the quadrature methods, or more simply with a planimeter. The amount of Ac-globulin of an unknown is determined by comparing the area of the unknown with that of a normal control. By referring the area to a standard reference curve, the Ac-globulin activity can be expressed in percent of normal. The prothrombin concentration is adjusted so that only small quantities of plasma are required for the measurement of Ac-globulin, thereby greatly minimizing the effect of antithrombin on the conversion rate and thrombin yield. A concerted effort was made to devise a one-stage technic for the quantitative determination of Ac-globulin. The results of such an attempt were unsatisfactory because the three variables, noted above, could not be isolated, and no one variable could be interpreted as being specifically related to Ac-globulin activity.

*Summary.* The chemical and/or physical relationship between prothrombin and Ac-globulin apparently is very close as evidenced by the inability to denature, adsorb, precipitate or otherwise inactivate completely, and at times partially, the one, without completely or partially removing or inactivating the other. A method is described whereby oxalated bovine plasma may be rendered free from Ac-globulin, but not from prothrombin.

Received March 7, 1950. P.S.E.B.M., 1950, v74.

## Relation of Oxygen to Photoreactivation of Bacteria After Ultraviolet Radiation.* (17800)

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The loss of capacity for cell division resulting from exposure to ultraviolet radiation has been shown in some instances to be restored by subsequent illumination with visible light

* This study has been aided in part by a grant from the American Cancer Society, through the Committee on Growth, of the National Research Council, and in part by an Institutional Grant from the New Jersey Section of the American Cancer Society to the Department of Biology, Princeton University, for fundamental biological research.

(1-5). Since the mechanism of this "photoreactivation" remains obscure, it has seemed important to test the possibility that photodynamic action, a process requiring molecular oxygen(6), is involved. The data reported herein show that $O_2$ is not essential, thus eliminating this possibility.

*Methods.* Washed cell suspensions in 0.5% NaCl solution buffered with 0.1 M $KH_2PO_4$ - $Na_2HPO_4$, usually containing about 1,000 cells per ml, were exposed to radiation from an intermediate pressure mercury arc through a Coret D filter. The intensity was approximately 1 x $10^4$ ergs/cm$^2$/sec. of wavelengths 0.27 $\mu$ to 0.313 $\mu$; the method of dosage is described by Blum and Price(7). In the sea urchin's egg, complete recovery of rate of cell division occurs after delay by such dosage (4,7). For photoreactivation suspensions were illuminated in Thunberg tubes placed at a distance of 12 inches from a bank of four 20 watt 'fluorescent' lamps ('Daylight' type). The reactivation is brought about by wavelengths in the blue, violet and near ultraviolet(1-4), in which the fluorescent lamps are relatively rich. Aliquot portions of the irradiated cell suspension were transferred to a series of Thunberg tubes arranged in pairs. One tube of each pair was wrapped in black cloth and placed adjacent to the corresponding illuminated tube to serve as a 'dark control'. Tubes were withdrawn a pair at a time at intervals during illumination and plate counts made from each tube. The influence of oxygen was determined in similar manner except that of each pair of tubes one was evacuated. Both then were illuminated and plate counts made. The virtually complete removal of oxygen from the evacuated tubes was indicated by the extinction of luminescence in a dilute suspension of *Photobacterium phosphoreum* connected to the same vacuum line. Bacterial luminescence is a particularly sensitive indicator for the presence of molecular oxygen(8).

*Results.* The photoreactivation of the facultative anaerobe, *Escherichia coli,* strain B, is illustrated in Fig. 1 by a typical experiment. Reactivation was not found to be very sensitive to temperature in the range of 22° to 37°C, and was about the same whether the hydrogen ion concentration was acid or near neutrality.

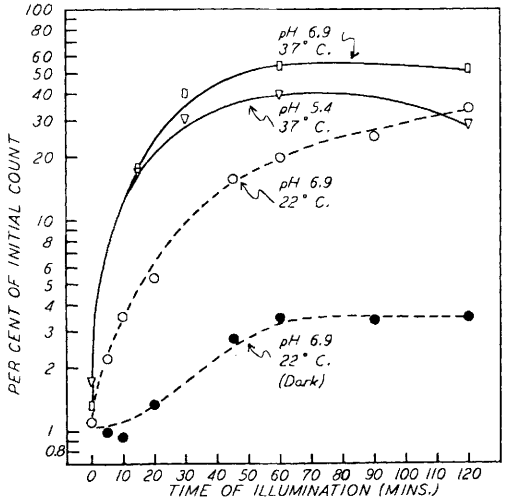


FIG. 1.

Percent of initial count of a suspension of *E. coli* after exposure to ultraviolet radiation ($\sim$ 2.5 $\times$ $10^6$ ergs/cm$^2$; $\lambda$ = 0.270 $\mu$ to 0.313 $\mu$), and subsequent illumination with visible light† for various lengths of time (open symbols, upper 3 curves) and in the dark (solid disks, lowest curve). Broken and solid lines refer to 2 different cell suspensions, each containing 880 cells per ml before exposure to ultraviolet radiation, the former illuminated at 22°C and the latter at 37°C. The pH of the salt solution was 6.98 in all except that represented by the next to uppermost curve (triangles) which was 5.4.

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† Zero time refers to the time at which illumination with visible light was begun. A few minutes elapsed between the end of ultraviolet irradiation and the beginning of illumination, during which all specimens were kept in the dark.

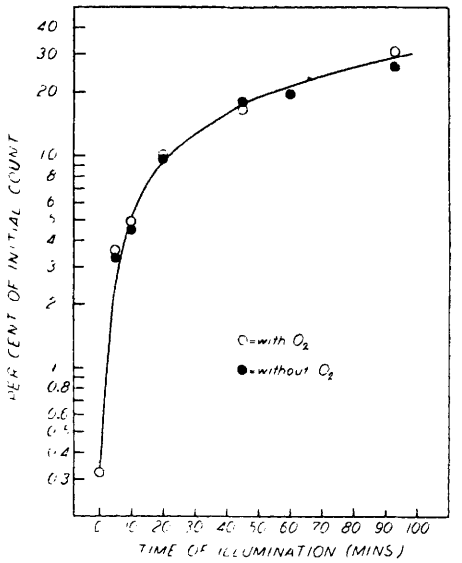


FIG. 2.

Photoreactivation† of *E. coli* in presence and absence of $O_2$, after exposure to ultraviolet radiation ($2.5 \times 10^6$ ergs/cm$^2$; $\lambda = 0.270\ \mu$ to $0.313\ \mu$). Initial plate count before irradiation was 1,100 cells per ml; pH of the salt solution was 6.9; temperature during illumination, 22°C.

That photoreactivation takes place in the virtual absence of molecular oxygen is demonstrated by the data in Fig. 2. The close agreement of the counts indicates that photoreactivation is completely independent of $O_2$. Dulbecco[3] has recently observed that photoreactivation of bacteriophage, in the presence of the host cells, is also independent of molecular oxygen, supporting the conclusion that photodynamic action is not the mechanism involved.

Efforts to study the same phenomenon in a more strictly aerobic organism, *Bacillus cereus*, were unsuccessful because no appreciable photoreactivation could be demonstrated. Typical data are illustrated in Table I. In these experiments, suspensions of wholly vegetative cells were obtained by inoculating glucose nutrient agar plates with spores and washing the cells after 5 hours incubation at 37°C. Microscopic observation confirmed that spore germination had been complete, and new sporulation had not begun. It will be noted that considerably higher dosages of ultraviolet were required to reduce the plate count than in the case of *E. coli*, and that subsequent illumination caused no significant increase. Thus although photoreactivation

TABLE I.

Influence of Illumination by Visible Light, on Plate Counts of *Bacillus cereus* After Exposure to Ultraviolet Light. Vegetative cells from 5 hour cultures on 1% glucose, nutrient agar plates at 37°C, washed and resuspended in 0.5% NaCl solution buffered at pH 7 ± .02 by 1 M $KH_2PO_4$-$Na_2HPO_4$. Suspension A, irradiated after two days' storage in the refrigerator; B, same suspension irradiated after 6 days; C, different suspension, after 3 weeks' storage in the refrigerator.

| Initial No. of cells per ml | Dosage ultraviolet radiation ergs/cm$^2$ | Plate count shortly after exposure to ultraviolet radiation | % initial count | Time of standing† (min.) | Illuminated | | Dark | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Plate count | % initial count | Plate count | % initial count |
| A. 5060 | $6.25 \times 10^6$ | 505 | 10.0 | 5 | 526 | 10.4 | 588 | 11.6 |
| | | | | 10 | 374 | 7.4 | 465 | 9.2 |
| | | | | 40 | 288 | 5.7 | 367 | 7.3 |
| | | | | 80 | 177 | 3.5 | 101 | 2.0 |
| B. 1490 | $6.25 \times 10^6$ | 199 | 13.4 | 5 | 9 | 0.6 | 44 | 2.9 |
| | | | | 10 | 69 | 4.6 | 126 | 8.4 |
| | | | | 20 | 8 | 0.5 | 30 | 2.0 |
| | | | | 40 | 10 | 0.7 | 14 | 1.0 |
| C. 180 | $2.7 \times 10^6$ | 10 | 5.5 | 5 | 8 | 4.4 | * | * |
| | | | | 10 | 7 | 3.6 | 7 | 3.6 |
| | | | | 20 | 11 | 6.1 | * | * |
| | | | | 30 | * | * | 10 | 5.5 |
| | | | | 40 | 11 | 5.9 | * | * |
| | | | | 80 | 10 | 5.5 | * | * |
| | | | | 95 | 8 | 4.4 | 3 | 1.7 |

* Not plated.

occurs in diverse types of cells and is probably a widely distributed phenomenon, it is not always readily demonstrable.

*Summary.* Molecular oxygen is not essential for the photoreactivation of *E. coli* after inactivation by ultraviolet radiation, demonstrating that photodynamic action is not involved.

Vegetative cells from young cultures of *Bacillus cereus* show no appreciable photoreactivation.

Received March 7, 1950. P.S.E.B.M., 1950, v74.

## Comparative Action of Aureomycin, Chloromycetin, Neomycin, Q-19, and Polymyxin B Against Gram Negative Bacilli. (17801)

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Although the number of effective antibiotics continues to increase, infections due to gram negative bacilli still pose many therapeutic problems. In the present study, the sensitivities of strains of *Pseudomonas aeruginosa, Aerobacter aerogenes, Escherichia coli* and *Proteus vulgaris* to neomycin, Q-19, polymyxin B, aureomycin, and chloromycetin have been determined. Streptomycin-resistant variants of *E. coli* have been compared with the resistant variants of the same strain appearing with neomycin and Q-19. Studies on the mode of action of neomycin and Q-19 are also presented.

*Materials and Methods:** Neomycin, produced by a member of the genus *Streptomyces* and discovered by Waksman(1,2), has been shown to be active against a wide variety of both gram negative and gram positive organisms. Q-19,† produced by a strain of *Bacillus circulans* and considered to be related to the polymyxin-aerosporin type of antibiotic was discovered by Murray and Tetrault(3). They reported it to be more active against gram negative than gram positive organisms. The activity of preparations from *Bacillus polymyxa* and a related organism, *Bacillus aerosporus,* against gram negative organisms was reported almost simultaneously by 3 groups of workers(5-7). A crude preparation of polymyxin B prepared by the Pfizer laboratory was used in this study. Aureomycin and chloromycetin, whose properties and effective bacterial spectra have been recently reviewed, were used as standards of comparison for the newer antibiotics(8-10).

Neomycin, Q-19, polymyxin B, and aureomycin were stable and readily soluble in concentrated solutions of sterile saline. Fresh stock solutions containing 10 to 25 mg per

* Neomycin and Q-19 supplied by Upjohn Co. Aureomycin supplied by Lederle Laboratories. Polymyxin B supplied by Pfizer Corp.

† Q-19 has also been referred to as circulin. It is a different antibiotic than the one described by McLeod(4), which was originally named circulin but has since been renamed polypetin.

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