determined by comparing the mortalities observed following graded doses given to comparable groups of mice.

Fig. 1 presents the composite data of *in vivo* experiments in which graded doses were given of both crude and pure benzyl penicillin preparations dissolved in sesame oil and of a crystalline water-soluble salt of penicillin (1,600 μ/mg) suspended in sesame oil. From this log-probit graph[3] the relative potencies of the 3 preparations as well as the efficiency of the two routes of administration may be estimated. In some of these tests the sodium penicillin was dissolved in an aqueous buffer solution, and it was observed that injections of sodium penicillin suspended in oil afford the same protection as the same amounts injected in buffer. When injected subcutaneously, the pure ester appears at least 3 times as potent as the pure salt on a weight basis, although some reservations must be made for a lack of parallelism in the straight lines relating dose to the degree of protection. If allowance is made for the difference in molecular weights the ratio becomes still greater. In other results not shown in the figure, the pure benzyl ester has been found to be 7.5 times as potent as an equimolecular amount of commercial sodium penicillin.

Of great interest also is the effectiveness of benzyl penicillin by mouth. In mice, as may be seen from Fig. 1, about 10 times as much of the crude benzyl penicillin (50 per cent. pure) was required orally as subcutaneously. A less pure preparation (10 per cent.) was better utilized by mouth, since only 4 times as much of it was needed for protection equivalent to that obtained from subcutaneous injections. The mouse appears to absorb water-soluble penicillin quite efficiently, since only three times as much (of either the pure or commercial grade salt) is required by mouth as subcutaneously. It should be noted that the mouse is a poor subject for oral administration experiments because of the relatively rapid passage of the drug through its intestinal tract. This situation favors the quickly absorbed water-soluble penicillin, since there is less exposure to adverse conditions in the stomach, but diminishes the action of the ester, which appears to require digestive cleavage before absorption.

The data presented above indicate that when injected subcutaneously in mice, benzyl penicillin is at least 3 times as potent as ordinary sodium penicillin in aqueous solution or suspended in oil. When taken by mouth, the benzyl penicillin is less active than by injection, but still is sufficiently potent to make it substantially as effective as an equivalent weight of sodium penicillin given by subcutaneous injection. The potential advantages of these favorable properties are obvious. Clinical data are being published elsewhere[4] which demonstrate that these advantages may also be seen in patients.

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THE FUNCTIONAL PATHOLOGY OF FROSTBITE AND THE PREVENTION OF GANGRENE IN EXPERIMENTAL ANIMALS AND HUMANS[1]

THE functional pathology of frostbite has thus far been obscure. Apart from the excellent studies of Greene,[2] who has approached this problem mainly from the morphologic viewpoint, few basic facts were available. The use of the fluorescein test[3, 4] has thrown more light on the pathologic physiology of the frostbite lesion. In this test small amounts of fluorescein are injected and its migration with the blood stream and into the interstitial spaces is observed under ultra-violet light.

Six rabbits were depilated on the abdomen and exposed to cold by applying the bottom of small glass beakers filled with dry ice to the skin. The periods of exposure varied from 5 to 90 minutes. Under this exposure the skin freezes solid and thaws after intervals varying between 5 and 25 minutes, depending on the length of exposure. For periods of 30 to 120 minutes following such refrigeration no fluorescein appears in the exposed areas indicating a severe spasm of the arterioles. After this time a second stage is initiated during which all blood vessels reopen and fluorescein can be seen throughout the exposed area. The diffusion of fluorescein into the surrounding tissues in the second stage is many times greater than in the non-exposed skin giving the picture of intense hyperfluorescence in the previously frozen areas. This period is also characterized by marked swelling of the exposed areas. Eight to fourteen hours after exposure a repeat fluorescein injection shows that now the exposed spots are not fluorescent, indicating a pre-gangrenous state. This non-fluorescence increases in the next hours until finally the entire spot is non-fluorescent and becomes gangrenous. Biopsies taken at this time show that, in agreement with the findings of Greene[2] and Krey-

[1] Aided by grants from the John and Mary R. Markle Foundation and the Council on Pharmacy and Therapeutics of the American Medical Association.

[2] R. Greene, *Jour. Path. and Bact.*, 55: 259–267, 1943.

[3] K. Lange and L. J. Boyd, *M. Clin N. A.*, 26: 934–952, 1942.

[4] *Idem, Arch. Int. Med.*, 74: 175–184, 1944.

berg,[5] there is a clumping of red cells in the smaller vessels which is probably due to loss of plasma through the highly permeable vascular wall. The red cells are stranded and silt the blood vessels forming a sludge. They do not, however, represent *true* thrombi in the beginning. A simple injection of saline enables one to wash out these erythrocytes as individual cells. Only after approximately 72 hours does organization of these cells into thrombi occur. This thrombosis ultimately leads to gangrene.

It appeared obvious therefore that therapeutic attempts to avoid gangrene after frostbite must be started before the stage of thrombosis is reached.

Ten rabbits of equal weight were exposed to chilling by the method already described. Five of the animals were treated by heparinization within four hours after exposure.[6] None of the heparinized animals developed gangrene, while in the untreated controls all areas exposed for more than 15 minutes became gangrenous. Encouraged by this result, the following experiment was done on 22 rabbits. One hind leg was exposed to an alcohol dry ice bath of −12°–20° C. for a period of 45 to 90 minutes with the leg protected by a thin boot of condom rubber. After the exposure, eleven animals were heparinized, while eleven remained untreated. Of the treated animals only two showed some slight surface lesions, while the legs of the others remained completely intact with no gangrene. All controls lost their legs by complete gangrene, including the bone.

The practical demonstration of the therapeutic value of heparinization in the prevention of gangrene was made possible by the study of artificial frostbite in human volunteers. These volunteers were recruited from patients who were being treated for subacute bacterial endocarditis at the Jewish Hospital of Brooklyn by the combination of penicillin and heparin.[7, 8] In one group the frostbite was accomplished by means of a porcelain crucible filled with dry ice and applied to the skin of the lateral aspect of the upper arm without pressure for ten minutes. An area of about 2 cm came in contact with the skin. By this method, a temperature of minus 22° C. was achieved. Heparinization was started immediately following exposure. One volunteer served as a control. The other group was subjected to local refrigeration in the same manner but for two exposures of 30 minutes each. The initial or control exposure was permitted to develop for six days before the second frostbite was induced, immediately following which treatment with the subcutaneous heparin in the Pitkin menstruum was initiated.[9, 10] The 30-minute exposure with dry ice results in temperatures considerably below minus 22 degrees Centigrade, and is comparable to the frostbite suffered by aviators in high altitude flying such as nose gunners after demolition of the plexi-glass protection or gunners attempting to un-jam guns without glove protection.

The clotting time in the treated cases stayed between 25 to 60 minutes. It was apparent from the observations in these human volunteers that all the adequately treated lesions escaped any deeper injury. One must recognize that these investigations in human volunteers are merely transition experiments which serve as added proof of the genesis of gangrene following frostbite and the validity of the therapeutic approach.

The further practical demonstration of the method's value was made possible when a frostbite case appeared at the Research Unit of the New York Medical College. A man was sent to the hospital following exposure to a temperature of 18 to 20 degrees F. for at least 14 hours while lying in the street. His hands were completely unprotected, while his feet were protected only by low shoes and thin socks. On admission his feet were ice-cold up to the knee and remained so for five hours after admission. He was heparinized by the intravenous route for five days, the clotting time being maintained between 30 and 60 minutes. He developed considerable blistering, especially on the hands, but completely escaped any permanent tissue loss. From the experience with similar exposures, one can say that this man without heparinization would probably have had more or less extensive loss of the extremities.

Experiments are under way to determine the simplest method of heparinization and the longest interval between exposure and start of therapy which would still be effective.

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ACTION SPECTRUM FOR THE PHOTOPERIODIC CONTROL OF FLORAL INITIATION IN BILOXI SOYBEAN

THE effectiveness of light applied to Biloxi soybean leaves during the middle of the dark period to prevent

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