collagen is found in the epidermis. In a kilo of skin, therefore, 546 g may be allocated to the connective tissue and 454 g to the cells of the skin and their surrounding ultrafiltrate. The numerical amount of water, chloride, and sodium that can be assigned to the ultrafiltrate volume cannot be satisfactorily estimated on the basis of what we know now; nevertheless, the low concentration of potassium (13.5 mM) suggests that the amount of the intracellular phase of the skin is small; and if magnesium is ascribed to the intracellular fluids of a tissue, the low concentration found in the skin (2.13 mM) should further corroborate this small intracellular phase.

Although the total water of the extracellular phase cannot be calculated at this time, the calculated 330 g of water identified with the 546 g of connective tissue per kilo of fat-free skin demonstrates that the volume of available water is considerably larger in the skin than any other tissue of the body. This finding corresponds to the results obtained by Flemister[13] for his work on mice.

Summary. Total water, nitrogen, collagen nitrogen, and electrolyte concentrations were determined in human skin which was removed at once from amputated parts during the course of surgery. The total skin (corium plus epidermis) gave the mean average results which are expressed as units per kilo of fat-free skin: total water, 717.7 ± 20.1 g; chloride, 79.9 ± 4.8 mM; sodium, 93.0 ± 8.0 mM; potassium, 16.47 ± 3.36 mM; calcium, 2.68 ± 0.52 mM; magnesium, 2.13 ± 0.30 mM; total nitrogen, 45.5 ± 3.8 g; and collagen nitrogen, 33.7 ± 4.5 g.

By applying methods now accepted as valid for calculating the weight of connective tissue in a tissue, the weight of connective tissue in human skin was estimated to be 546 ± 73 g per kilo of fat-free skin. The calculated 330 ± 45 g of water identified with this amount of connective tissue demonstrates that the volume of available water is considerably larger in skin than in any other tissue of the body. The skin may be regarded as one of the most important water-storage organs of the body.

We wish to express our appreciation to various members of the Department of Surgery for making the tissues used in this study available to us.

[13] Flemister, L. J., *Am. J. Physiol.*, 1942, **135**, 430.

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Effect of Penicillin on Experimental Staphylococcus Osteomyelitis in Rats.*

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Experimental staphylococcal infections in animals which simulate natural occurring infections in man are difficult to produce. In order to evaluate the recently introduced chemotherapeutic agents it would be desirable to have access to readily reproduceable *in vivo* procedures. The purpose of this study was to develop methods for the production of chronic staphylococcal infections in mice and determine the value of penicillin in the treatment of such infections.

With economy of penicillin in mind, several animals were considered, and on investigation the rat was found to be suitable for the purpose. The production of osteomyelitis in this animal did, however, present a major problem which was quite distinct from that of treatment. Because of the nature of the problem

* The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

and the extent of the experimental work involved, it is deemed desirable to report this study as a separate publication.

Experimental Work. The method of producing the infections finally employed was as follows: Albino rats, Wistar strain, weighing 50 to 100 g, were anesthetized with nembutal, and a small hole (about 2 mm diameter) drilled into the bone from the medial surface at the proximal end of the tibia. With the aid of curved forceps, a pledget of cotton soaked with a 24-hour broth culture of *Staph. aureus* was inserted into the bone marrow. The strain of organism used was originally isolated from a patient with chronic osteomyelitis of hematogenous origin. The skin was then sutured with No. 50 white cotton thread.

After a period of from 21 to 26 days, the surviving animals were again anesthetized, the cotton pledgets removed and the character and extent of the lesions observed. Cultures were made by inoculating proteose peptone broth with material from deep in the involved area, and incubated at 37°C for 24-48 hours. When growth occurred, smears were made and stained by Gram's method.

A total of 80 animals was inoculated; 70% developed osteomyelitis, 19% died during the developmental period, and in 11% the initial lesions healed uneventfully. To be sure that the desired infection had been produced, material from 14 of the animals was examined histologically† and found to be acute or chronic osteomyelitis in each instance.

Two experiments were performed to test the effect of penicillin. The treatment was instigated promptly following the establishment of typical infections. In the first experiment, each of 14 rats was given 600 units of the sodium salt of penicillin subcutaneously every 2 hours for a period of 9 days, a total of 64,800 units. Fifteen animals received no drug and served as a control. Two hours after the final injection of penicillin, samples of blood were obtained by cardiac puncture and the penicillin content determined by the Oxford cup method as described by Abraham *et al.*[1] At this time the blood level was found to be consistently less than 0.1 unit per ml. Gross examinations revealed that of the 14 treated animals, 5 had lesions which had decreased in size and appeared to be healing, 3 showed no change, and 6 had apparently recovered, *i.e.*, the lesions had either completely healed or reduced in size and contained sterile pus. In the control group the lesions had progressed in every instance but one in which spontaneous recovery had apparently taken place. Cultural material from all animals in which recovery had not occurred was positive for staphylococci.

It appeared that the penicillin had exerted a beneficial effect. Since the blood content of penicillin dropped to a very low level between injections it was thought that larger amounts given at more frequent intervals might produce more favorable results.

In a second experiment the methods were essentially the same except that the dosage of the drug was increased. Each of 13 animals received 900 units of penicillin every hour, instead of 600 units every 2 hours, and, therefore, a total of 194,400 units during the 9 days. One hour after the final injection the blood level was determined and again found to be consistently less than 0.1 unit per ml. Cultures of this blood showed the presence of staphylococci in all animals including the controls. On macroscopical examination the lesions in 6 of the treated rats had healed, in 3 there was no suggestion of change, and in 4 there was a more extensive involvement. On culturing material from the original areas of inoculation, the six in which healing had occurred gave no growth, even though blood cultures from these animals were positive for staphylococci. The specimens from the other 7 treated animals all gave positive cultures of staphylococci. Of the 10 control animals in this experiment, none showed any evidence of recovery and pus from all yielded positive cultures of staphylococci.

Discussion and Conclusions. The data of the above experiments indicate that penicillin does exert a beneficial effect on experimental

† Kindly examined by Dr. Carl V. Weller of the Department of Pathology.

[1] Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **2**, 177.

osteomyelitis in rats. The fact that the blood level of the drug drops very rapidly following the injection of such heavy doses led to efforts to maintain higher levels by simultaneously injecting other materials designed to reduce kidney excretion or to decrease the rate of absorption. No satisfactory method was found. It is of interest to note, however, that Romansky and Rittman[2] have recently reported such an accomplishment by mixing penicillin with beeswax and peanut oil. Although they report no development of sensitization to these accessory substances, this has not been the experience of others. The fact that the 900 units per hour produced no better results than 600 units every 2 hours, plus the low blood levels found following the final injections, indicates that if some method of maintaining a nearly constant level were used, the level could be rather low and still produce good results. Since staphylococci were consistently found in the blood, even after the lesions had healed, it seems that the treatment should be continued for a greater period of time.

These results indicate that penicillin has definite value as a systemic therapeutic agent for staphylococcus osteomyelitis in rats; and further, that the rat is a suitable animal for studying the course of experimental osteomyelitis, and the evaluation of therapy in this disease.

Summary. Osteomyelitis was produced experimentally in white rats by inoculating the bone marrow of the tibia with *Staph. aureus*. About 21 days were allowed for the development of typical lesions. One group of 14 animals was treated by the subcutaneous injection of 600 units of penicillin every 2 hours for 9 days. Six of these apparently recovered during the course of the treatment. In a second experiment 13 animals were each given 900 units of the drug every hour for an equal period of time. Again 6 of the lesions were found to be healed at the termination of the injections. Of a total of 25 untreated controls only one animal showed evidence of recovery. It is concluded that penicillin has value as a systemic therapeutic agent in the treatment of the experimental disease, and that the rat is a suitable animal for the study of the course of experimental osteomyelitis and the evaluation of therapy.

[2] Romansky, M. J., and Rittman, G. E., *Science*, 1944, **100**, 196.