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PYOGENIC OSTEOMYELITIS: DIFFUSION IN LIVE AND DEAD BONE WITH PARTICULAR REFERENCE TO THE TETRACYCLINE ANTIBIOTICS*

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Short, intensive courses of antibiotic therapy enjoy considerable success in the treatment of soft tissue pyogenic infections. It has been learned empirically that such therapy is seldom adequate in the treatment of pyogenic osteomyelitis, which requires higher doses and longer treatment to reduce recurrences to acceptable levels.[2,5,7] The reasons for these differences are not well known and as a result the advent of each new antibiotic is accompanied by sporadic, unsuccessful attempts to cut short the antibiotic treatment time in cases of pyogenic osteomyelitis. It has been pointed out previously that a bacterial reservoir exists in the one million-plus spaces present in each cubic millimeter of infected bone.[17] This paper considers diffusion processes which affect the ability of administered antibiotics to reach the bacterial reservoir of pyogenic osteomyelitis.[a]

At present it is not possible to treat diffusion process in bone mathematically or even with experimental precision. The reasons for this state of affairs become apparent when the list of factors known to affect diffusion in ideal systems is reviewed. This paper accordingly will present and interpret the results of some *qualitative* experimental work done on living and dead human bone. The tetracycline antibiotics are at the core of our experimental methods because of their convenience, their fixation in bone in vivo,[16] the availability of human bone specimens from a sizable number of patients who received the drugs during life and because of the ease with which they may be detected in very minute amounts by available methods. The methods evolved in this laboratory have the virtue of simplicity and economy as well as the sensitivity of other methods.[15,16]

In order to tell whether diffusion behavior of the tetracycline antibiotics is a special case or if their behavior is generically representative a group of other reagents have been compared to the tetracyclines in bone in vitro. The results of such experiments are summarized. We feel that, in the absence of suitable accurate measuring methods, a detailed presentation of our experiments would constitute needless verbosity. Our data are based on qualitative, visual observations.

FACTORS AFFECTING DIFFUSION IN ISOLATED SYSTEMS[3,6,8,19,20,24]

We are concerned with the diffusion of solute molecules in the extracellular fluids in the spaces of human bone. It has been pointed out elsewhere that diffusion through mineralized bone matrix does not occur in molecules exceeding the size of glucose or similar organic substances. If we consider *diffusion impedence* to be resistance to motion of a solute particle from one point to another in the solvent, we may then list factors known to *increase* diffusion impedence, first in a limitless volume

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(a) The bacterial reservoir is the bacteria harbored in the spaces (lacunae, canaliculae, Haversian and Volkmann's canals and medullary cavity) found in bone.

of solvent, and second along a finite column of solvent bounded by a container whose walls may exhibit specific effects on diffusion impedence.

In a limitless volume of solvent, diffusion impedence is increased by: increase in pressure; decrease in temperature, solute concentration, mean free path; increase in mass and charge of solute and solvent ions; specific interaction effects of one solvent molecule with another, one solute molecule with another and of solvent with solute; by adventitious solutes; by specific effects of pH on solvent and solute; by increase in size of solute and solvent molecules.

In tubular lumens diffusion impedence is increased by: decreasing the radius of the tube lumen (canalicular radius is 200 millimicrons) increasing the length of path from one point to another (as occurs in tortuous tubes); decreasing the total cross section area through which diffusion occurs (about 8 square microns of canalicula connect each lacuna to a vascular channel while the average cross section area of an Haversian canal is 0.005 mm^2); interaction between the wall of the lumen on the one hand and the solutes and solvent on the other; specific effects of pH, dielectric constant of the solvent, solute and wall of the lumen; and finally by the presence of a gel state (a specific interaction effect normally present in biological systems) filling the lumen in which diffusion occurs. This listing is not complete.

It may be seen that quantitatively this is a hopeless problem for the present!

DIFFUSION IN BONE *IN VITRO*

The sum of the following material is that bone is highly impermeable and that diffusion processes in its fluid-filled spaces are normally highly impeded. The impedence "wall" is normally surmounted by energy supplied by living, metabolizing cells.

Our in vitro work was done on fresh human bone. By fresh we mean the bone was obtained and sectioned less than 6 hours after death or surgery. Bones investigated include rib, clavicle, femur, tibia, fibula, radius and ulna. There were no consistent differences among them. The sections referred to subsequently were undecalcified, unfixed, unembedded, undehydrated, thin sections made by Frost's method.[9,10] This method introduces less artifact into bone than any other existing method. Lo Grippo and Hirsch have kept human biopsy specimens prepared by this method alive in special media for periods as long as 6 weeks.[21]

We soaked fresh sections 60 - 70 microns thick in the following reagents: aureomycin, achromycin, terramycin, $\overset{+}{Ag}$, $\overset{++}{Hg}$, $\overset{++}{Pb}$, $\overset{++}{Cu}$, $\overset{++}{Co}$, $\overset{++}{Fe}$, $\overset{+++}{Mn0_2}$, $\overset{+++}{Cr_2O_7}$, $\overset{-}{I}$, $\overset{-}{F}$, $\overset{-}{Br}$, glucose, urea, various dyes such as acid and basic fuchsin, methylene blue, gentian and crystal violet, toluidine blue, eosin G and Y, saffranin, several haematoxylins, chlorazol black, nile blue sulphate, Sudan III and IV and india ink. Inorganic substances were subsequently demonstrated as colored inorganic precipitates. The dyes were directly visible in situ. Reagents such as glucose, urea and citrate were added to other reagents or used before them to detect blockade, diffusion impedence or bone diffusion phenomena of specific nature.

In aqueous solutions the majority of the above reagents are very poor permeators

of the canaliculae and lacunae in the center of the bone sections over time periods of 2 - 7 days at physiological temperatures and pressures. Concentrations were on the order of 0.1 Molar, except where solubility considerations prohibited. Where it could be investigated the effct of pH over the range of 6.5 - 8.5 was tested with no effect on diffusion impedence noted. Acetate, citrate, carbonate and phosphate buffer systems were used. The specific ion effects need not be considered here. It must be noted that our methods are crude and only rule out a major pH effect on diffusion impedence. It would be astonishing if pH had no effect at all.

We could improve permeation radically by the following: drying sections 4 hours at 40° C before staining, use of 40% ethanolic solutions, and boiling in the reagent. Boiling produces severe, irreversible permeability defects which the other two methods do not.

It is known in life that some osteocyte lacunae are normally empty, the percentage of empty lacunae increasing with age;[12] that in most elderly patients a peculiar obliteration of canaliculae and lacunae occurs which is termed micropetrosis and may affect little or much of the skeleton;[11] and that a peculiar state of incomplete mineralization termed Feathering occurs in human bone, in some cases affecting more than half of the axial skeletal volume.[13] It has been pointed out elsewhere that a molecular size effect can be seen in bone which limits the size of molecules which can diffuse into the bone matrix and which might be expected to affect the exchange of substances between bone and blood.[14]

Our experiments offered us the opportunity to observe diffusion impedence phenomena in the disease states noted above.

In *in vitro* sections there is little difference in permeation of the lacunae and canaliculae between live and dead ones, although the live ones may have slightly better permeation. No diffusion into the canaliculae or matrix of micropetrotic bone has been observed in an extensive amount of material. Ready diffusion into feathered bone occurs.

A molecular size effect on diffusion impedence is clearly present. There are also erratic specific effects of unknown nature which will make a particular substance peculiar in its behavior—methylene blue for instance, a good permeator even in aqueous solutions; and ferric ion which even in dilute solutions decalcifies sections rather than permeates them.

No difficulty was experienced in staining the walls of the vascular channels in the sections used in the above work. We interpret this to mean lower diffusion impedence in these relatively large spaces. The ease of staining the walls of the vascular channels also indicates that the locus of highest diffusion impedence lies in the canaliculae, since these canaliculae open directly into the vascular channels.

When diffusion impedence is checked in fresh, bulk bone* it is found that penetration of all the vascular channels requires over 6 weeks even for moderately thin bones such as ribs, and that this can only be achieved with the use of ethanolic solutions. The job is hopeless with purely aqueous solutions.

*Cubes 1-2 cm. on a side.

We secured experimental evidence that increasing reagent concentration, increasing temperature, using smaller molecules and destroying the gel plug (which bacterial invasion of infarcted bone presumably also does) in vascular channels considerably lowered diffusion impedence.

DIFFUSION IN BONE *IN VIVO*

For these observations we are dependent upon the behavior of tetracyclines administered in vivo over variable periods of time but, in all the cases whose findings are quoted herein, administered continuously up to within a day of surgery or the time of death. Since the tetracycline antibiotics become permanently fixed only in mineralizing bone, and wash out of other tissues when dosage is stopped, the continuance of dosage to within 24 hours of death was a necessary requirement for the present observations.

Tetracyclines were detectable by microfluorescence methods in many but not all lacunae and canaliculae containing live cells after 2-5 days of continuous dosage, but not in the case of shorter dosage. Tetracyclines were usually not detectable in these spaces if the drug had been stopped 48 or more hours before death or surgery, the exception being an anuric, uremic patient who still had demonstrable tetracycline in lacunae 5 days after drug dosage was stopped.

Tetracyclines were not detectable in dead canaliculae or lacunae even after 2 weeks of continuous drug dosage, nor were they detectable in micropetrotic bone.

Tetracyclines were detectable in feathered bone in less than 10% of the feathered systems examined, indicating that some unapparent factors reduce the permeability of feathered bone in vivo.

Tetracyclines were detectable as stains of the walls of the vascular channels as expected but with an unexpected feature: a variable number of the channels were unstained in each section examined. We interpret this to mean that these channels did not contain patent blood supply during the period of drug administration. (In vitro *any* exposed bone surface can be stained with tetracyclines; the surface stain may also be removed at will).

DISCUSSION

General Aspects

Physical-chemical effects have to be invoked to explain the poor diffusivity of the reagents tested in human bone in vitro and in vivo. Effects on diffusion impedence of temperature, concentration, the size and structure of canaliculae, the protoplasmic plug in vascular channels, and size of the diffusing molecules have been observed. So many additional factors might affect diffusion impedence in vivo that we must consider the surface of the topic barely scratched. One outstanding fact emerges from our work, however, and this is the very high diffusion impedence normally found in dead bone, whether in vitro or in vivo.

This diffusion impedence is so high that the histologist may well ask how osteocytes can live in their bony prison. The explanation must be the metabolic pump.[14]

This pump is the system of concentration gradients produced and maintained by the chemical activity of the osteocytes. It is known that cells can concentrate (another way of saying create a concentration gradient) substances by factors of 200 to 10,000,000 and there is no reason to expect osteocytes to be exceptional in this respect.

We have obtained clear evidence that the diffusion of tetracycline antibiotics in dead bone is highly impeded. The similarity of a group of substances chemically unrelated to the tetracyclines makes it probable that high diffusion impedence affects other antibiotics used in the therapy of pyogenic osteomyelitis. Diffusion impedence is greatest in canaliculae. The failure of reagents to diffuse 100 microns along canaliculae in 48 hours or more indicates that some specific effects are present which create a diffusion barrier in the canaliculae.

The in vitro and in vivo impermeability of micropetrotic bone might have been anticipated in view of the nature of this bony change. The failure of most of the feathered bone present in a skeleton to stain with tetracycline in vivo is surprising in view of the ready permeability of feathered bone in vitro. One wonders if feathered bone is "available" to the blood for exchange of the much smaller, more mobile electrolytes and buffer ions. It is possible that some peculiar attribute of feathered bone or its cells makes it less active physiologically than normal bone.

PYOGENIC OSTEOMYELITIS

A bacterial reservoir occurs in osteomyelitic bone.[17] The bacterial reservoir is composed of bacteria harbored in the myriad spaces normally found in bone. These bacteria in some cases are able to remain in viable, potentially infective states for many years and are the usual source of infecting bacteria in a recurrence of pyogenic osteomyelitis.

Diffusion behavior probably explains the failure of short periods of antibiotic treatment to kill all the bacteria in the osteomyelitic bacterial reservoir. The high diffusion impedence to large molecules found in dead bone in vitro and in vivo prevents antibiotic permeation of dead, infected bone in short time periods.

From general knowledge of the pathology of pyogenic osteomyelitis[1,4,18,25] and the work presented here it is possible to explain some aspects of the disease.

First, small portions of dead bone surrounded by good blood supply might be sterilized in 6 to 8 weeks by the proper dosage of antibiotic. The larger the portions of dead bone become, however, the less complete will their permeation by the antibiotic be and the more likely it is that viable bacteria will remain in some of the bone spaces after discontinuance of the antibiotic. Large bone portions, such as the entire cortical thickness over a length of several centimeters of tibia, probably could not be thoroughly permeated with antibiotic even after a year of continuous dosage. This behavior explains the high recurrence rate in cases of pyogenic osteomyelitis in which for any reason institution of proper therapy was delayed. It is in just such cases that large portions of cortical bone die, due mostly to the effects of vascular thrombosis and increased intramedullary pressure.

Second, it should take several days of therapy to ensure life of infected but still living cells. These cells would die if the infecting organism were not subdued

Third, the desired rapid improvement in clinical signs and symptoms which follows administration of antibiotic cannot mean the obliteration of the bacterial reservoir. It means obliteration of the organisms in infected but viable soft tissue and bone. *The bacterial reservoir is a separate problem and its elimination necessitates long, high dosage therapy.*

The failure of antibiotics to sterilize large masses of infected bone gives experimental teeth to the clinical dictum that treatment should be begun when the disease is suspected. Waiting will result in death of larger and larger masses of bone.

Several things may be inferred from our work which may be summarized in the general statement that *it should be possible to tailor the physical chemistry and ordinary chemistry of an antibiotic to the treatment of pyogenic osteomyelitis.* Several attributes of such an antibiotic may be suspected in advance (due to the complexity of the biological system we are dealing with, these attributes must be tested in that system before they are accepted as valid).

Figure 1

Undecalcified cross section clavicle between crossed polarizers, revealing Haversian canals (large black ovals), Haversian systems surrounding the canals, and extrahaversian bone filling in around the Haversian systems. The channel at 6 o'clock is the end of a Volkmann's canal.
The Haversian vessels lie in the Haversian canals. These vessels are the normal source of substances brought to bone by the blood. To permeate the bone a substance must diffuse out of the vessels into the perivascular fluids in the Haversian canal: thence into the canaliculae and thence to the lacunae.

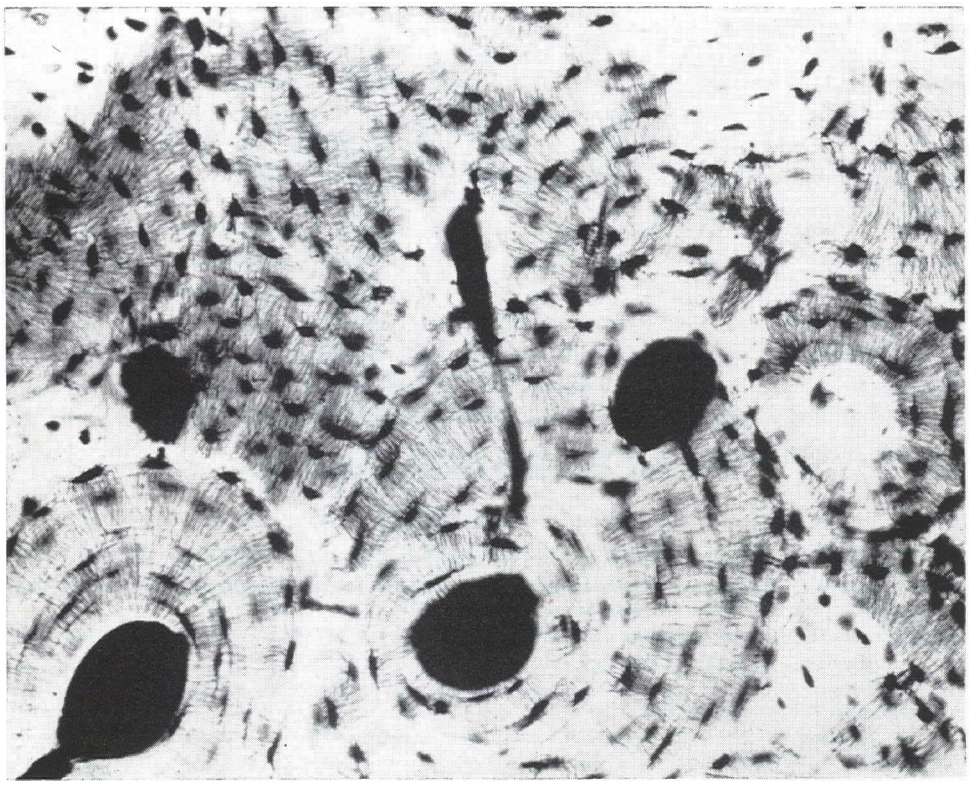

Figure 2

Undecalcified cross section femur, stained with basic fuchsin. Magnification and field selected to reveal the lacunae and their canalicular network. The function of canaliculae is to provide tubular lumens in the impermeable mineralized matrix connecting lacunae to sources of blood supply: Haversian canals.

First, an effective bone permeator should be of small molecular size. Second, it should have small net charge at the pH of the fluids in the bone spaces (this pH is not known). Third, and most promising, one or more specific interaction effects might be used to accelerate permeation of dead bone by the antibiotic. For example, one can speculate that the ability of the tetracyclines to complex with magnesium and calcium might be put to good use.

Theoretically an increase in pH of the body fluids bathing a child's dead bone might increase the rate of accretion of mineral in the dead bone.* If antibiotic molecules could be carried into the dead bone by this slow current of mineral a specific jumping mechanism surmounting the diffusion impedence barrier would be present. We hope the reader will excuse the details of our speculation and instead fasten on the possibility we are discussing: that antibiotics might be "tailored" chemically and physically for the task of treating ostemyelitis.

*Children's bones are on the whole less densely mineralized than adult's bones and are continually accumulating new mineral, although at slow rates. Mineral accretion is more rapid at more alkaline pH.

CONCLUSION

We believe the failure to sterilize large masses of infected bone in vivo with antibiotic agents has been explained by the high diffusion impedence normally present in bone as has the necessity for early diagnosis and treatment and prolonged treatment of pyogenic osteomyelitis. More important, we believe the work and thinking we have presented provides fertile ground for additional work on the several topics touched on in our presentation.

SUMMARY

Both in vitro and in vivo, dead bone is afflicted by a very high diffusion impedence which impairs the permeation of its myriads of tiny spaces by antibiotics. This impedence to antibiotic diffusion explains the failure of courses of antibiotic to cure pyogenic osteomyelitis when given for short periods of time or when given in the presence of large masses of already dead bone.

REFERENCES

1. Aegerter, E. E., and Kirkpatrick, J. A., Jr.: Orthopedic Diseases, Philadelphia, Saunders, 1958.

2. Altemeier, W. A., and Wadsworth, C. L.: Evaluation of penicillin therapy in acute hematogenous osteomyelitis, J. Bone & Joint Surg. 30A:657, 1948.

3. American Institute of Physics: Handbook, ed. by D. E. Gray, New York, McGraw-Hill, 1957.

4. Anderson, W. A. D.: Pathology, ed. 2, St. Louis, Mosby, 1953.

5. Beerman, C. A.: Treatment of acute hematogenous osteomyelitis of long bones in infants and children, J. Pediat. 33:578, 1948.

6. Goldacre, R. J.: Folding and unfolding of protein molecules as a basis of osmotic work, Internat. Rev. Cytol. 1:135, 1952.

7. Buchman, J.: Acute hematogenous osteomyelitis, Instruct. Course Lect. Am. Acad. Orthop. Surgeons 9:413, 1952.

8. Clark, W. M.: Topics in Physical Chemistry, ed. 2, Baltimore, Williams & Wilkins, 1952.

9. Frost, H. M.: Preparation of thin undecalcified bone sections by rapid manual method, Stain Technol. 33:273, 1958.

10. Frost, H. M.: Staining of fresh, undecalcified, thin bone sections, Stain Technol. 34:135, 1959.

11. Frost, H. M.: Micropetrosis, J. Bone & Joint Surg. 42A:144, 1960.

12. Frost, H. M.: In vivo osteocyte death, J. Bone & Joint Surg. 42A:138, 1960.

13. Frost, H. M.: New bone affection: Feathering, J. Bone & Joint Surg. 42A:447, 1960.

14. Frost, H. M.: Some aspects of the mechanics and dynamics of blood-bone interchange, Henry Ford Hosp. M. Bull. 8:36, 1960.

15. Frost, H. M.: An economical microfluorescence set-up for detection of tetracyclines in bone, Henry Ford Hosp. M. Bull. 8:197, 1960.

16. Frost, H. M.: In vivo staining of bone with tetracyclines, Stain Technol. 35:135, 1960.

17. Frost, H. M.: Pyogenic osteomyelitis: The normal bone spaces as a bacterial reservoir, Henry Ford Hosp. M. Bull. 8:263, 1960.

18. Howorth, M. B.: Textbook of Orthopedics, Philadelphia, Saunders, 1952.

19. Jacobs, M. H.: Diffusion processes, Ergebn. Physiol. 12:1, 1935.

20. Johnson, F. H., Eyring. H., and Polissar, M. J.: Kinetic Basis of Molecular Biology, New York, John Wiley, 1954, Chap. 14.

21. LoGrippo, G., Hirsch, L. J., and Frost, H. M.: Unpublished work.

22. Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 6, Philadelphia, Saunders, 1952.

23. Neuman, W. F., and Neuman, M. W.: Chemical Dynamics of Bone Mineral, Chicago, University of Chicago Press, 1958.

24. Randall, J. E.: Elements of Biophysics, Chicago, Year Book Publishers, 1958.

25. Weinmann, J. P., and Sicher, H.: Bone and Bones, ed. 2, St. Louis, Mosby, 1955.