grinding bacteria is not necessarily correct. If there is justification for using glass abrasives, it is because the adsorption of cellular material to the surface is less with glass than with other abrasives. However, Hyflo Super-Cel is believed to be quite free of adsorptive properties (*2*) and is easily prepared for use as an abrasive; it would thus seem more desirable than glass. Hyflo Super-Cel gives grinding efficiencies of 99.9% with 1 part cells, 2 parts abrasive, and 6 parts water.

Efficiency of grinding bacterial cells is chiefly dependent on the total abrasive action applied to the cells. In general, all techniques tried tended to support this concept. Evidence for this is exhibited by the higher efficiency of long grinding periods over short grinding periods. Additional evidence is the fact that the Wood-Werkman mill supplies more abrasive action than the Potter's homogenizer when pyrex glass is used as an abrasive. This is due to the gearbox and more powerful motor on the Wood-Werkman mill setup, which overcomes the friction of the glass paste more readily than the smaller motor on the homogenizer. When grinding large volumes of material and/or when using glass as the abrasive, the Wood-Werkman mill is more advantageous.

The two hardest abrasives tried, flint and pyrex glass, are not as efficient as the others, probably because of the coefficients of friction, as well as the character of the particle surfaces, and are more difficult to prepare. Levigated alumina as an abrasive gives efficiencies of 99% or better, with no lethal effect on prolonged contact with the cells in mixtures of these proportions:

a) 2 parts cells, 2 parts abrasive, and 1 part water
b) 1 part cells, 2 parts abrasive, and 1 part water
c) 5 parts cells, 20 parts abrasive, and 6 parts water

On the basis of viable cell counts, the Potter's homogenizer is an efficient grinding method to use for bacterial cells and possesses a capacity that yields adequate amounts of preparations for most experiments. It does not necessarily follow, however, that the resulting cell-free extracts will have high enzyme activity. The advantages over the Wood-Werkman mill are:

a) It requires less special equipment and storage space in the laboratory.
b) The initial cost of the material is a fraction of the cost of the Wood-Werkman mill setup.
c) It is more versatile and can be used with greater facility.
d) It is easier to refrigerate.
e) Sterile technique can be more easily applied.
f) Less material is lost in grinding, and smaller amounts can be used with satisfying results.
g) It offers easy modification to grind under anaerobic conditions simply by attaching a jet tube and flooding the upper portion of the tube with the desired inert gas.
h) High grinding efficiency can be obtained in less time.
i) Cell destruction can be obtained without addition of abrasive material.

The final trial of grinding without an abrasive in the Potter's homogenizer compared very favorably with the efficiency of the Booth-Green mill (*3*), which gives a 50% kill in 2 hr without refrigeration. The utility of grinding without an abrasive is questionable because of the long time required and the relatively low efficiency.

References

1. Umbreit, W. W., Burris, R. H., and Stauffer, J. F. *Monometric Techniques and Related Methods for the Study of Tissue Metabolism.* Minneapolis: Burgess Publ. Co. (1945).
2. Strain, H. H. *Chromatographic Adsorption Analysis.* New York: Interscience (1942).
3. Booth, V. B., and Green, D. E. *Biochem. J.*, **32**, (5) 855 (1938).

The Fluorine Content of Some Miocene Horse Bones

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In view of the recent successful application of fluorine analysis in determining the relative ages of various human and animal remains from the well-known Galley Hill and Piltdown sites, it occurred to the author that this method might advantageously be extended to fossil bones from still earlier horizons. The following paragraphs present a few such data obtained through analysis of Lower Miocene horse material from the Raeford Thomas farm, Gilchrist County, Florida.[1]

Inquiry into the fluorine content of both recent and fossil bone dates back at least to the beginning of the 19th century. The French mining engineer A. Carnot (*2*) has furnished an interesting account of these early procedures and determinations. In order to make their work quantitative, however, pioneers in this field were under the necessity of employing rather lengthy and tedious routines. The modern technique, a modification of which was applied to the British specimens, was published in 1933 by Willard and Winter (*3*). The method is both sensitive and specific. It is typical of the growing tendency to employ organic agents in the determination of inorganic ions. It has, moreover, the advantage, often inestimable to the anthropologist or paleontologist, of requiring only small amounts of tooth or bone, the analysis of which by ordinary gravimetric methods would entail a high percentage of error. In every essential it is the method that has been followed in the present analysis of horse remains.

Since the value of the test for this purpose depends upon its indication of relative, rather than absolute, amounts of fluorine, every effort was made to follow precisely the same routine in each trial of every sample and thus to secure consistent results. The bones were first

[1] Permission to examine these fossils was kindly accorded by A. S. Romer, director of the Museum of Comparative Zoology. The author also wishes to thank Arthur Loveridge, of the same institution, for calling his attention to the article by Kenneth P. Oakley on "Relative Dating of the Piltdown Skull" (*1*).

TABLE 1

FLUORINE ANALYSIS OF LOWER MIOCENE HORSE BONES

Sample No.	Description	No. titrations	Fluorine, %
1	large, light, unworn	3	2.4
2	" dark, "	3	1.7
3	small, light, "	3	2.6
4	" dark, "	3	1.9
5	large, light, worn	3	1.5
5a	" " "	1	1.5
6	" dark, "	4	1.7
7	small, light, "	5	2.7
8	" dark, "	3	1.7
9	large, light, "	1	1.9
10	" " "	1	1.9
11	recent	3	< 0.01

pulverized, and the material well mixed to ensure proper sampling of both dense and spongy tissue. To promote accuracy, the comparatively large amount of 1 g was used in each distillation. The latter was carried out with a measured volume of 60% perchloric acid under prescribed conditions of temperature and in the presence of soft glass (source of silica), the volume of each distillate being carefully noted. Aliquots of each were titrated with .10% solution of thorium nitrate, a fixed amount of the zirconium-alizarin indicator being used for each titration. It is necessary to make a small correction for the fluorine that combines with alizarin. The agreement between fluorine values calculated from successive titrations of distillate from a given bone was unexpectedly close. Average values only are reported in Table 1. They give the percentage weight of fluorine in the fossil. A necessary preliminary, of course, is to establish a working curve by titrating standards of known fluorine content (as fluoride) with thorium nitrate. Because none of these specimens was sensibly contaminated with matrix, the additional determination of phosphate has been omitted.

Briefly, the fossil bone quarry from which these Lower Miocene specimens were collected has yielded a large number of disarticulated elements, belonging, for the most part, to what has been described by T. E. White as a monophyletic series, *Parahippus blackbergi—P. barbouri—P. leonensis—Merychippus gunteri* (*4*). These bones appear to have been deposited in the channel of a stream, and some are plainly water-worn. The time interval is that represented by most of the Tampa and the lower part of the Hawthorne formation.

A study of their size relations (*5*) shows very clearly how these elements, kind for kind, sort into two size groups, the large and the small, the latter being less numerous. From the standpoint of preservation, they may again be separated as light in color or dark, worn or unworn. The skeletal unit chosen for analysis was the first phalanx (*os suffraginis*) of the principal digit, and the fluorine values listed for each of the descriptive categories in Table 1 refer to this bone alone.

It will be observed that the values fall into two groups: an upper, averaging 2.6%, and a lower averaging 1.7%. Their difference approximates the order of 1%, the total range being less than 1½ parts per hundred. The fluorine content is also seen to be independent of size. Although extreme values are found for light-colored bone, the dark furnishing low figures only, the data are considered insufficient to establish any correlation with iron content. In the case of the Piltdown and other British materials, this correlation was reported as inappreciable (*1*).[1] Most significant of all, perhaps, is the circumstance that both low and high values occur irrespectively in worn and unworn bone.

For the sake of comparison, a figure is included in the table for the fluorine content of recent bone. The element is reported present in human bone in amounts ranging from 0.01 to 0.03%. The author's analysis of an ashed sample from the first phalanx of *E. caballus* yielded a value less than 0.01%. These figures at least serve to emphasize one specific difference between recent and fossil bone.

Under circumstances where the principle of fluorine-dating would apply, so marked a difference between these groups of values might properly be taken to indicate two origins in time. However, the above-stated and well-attested time interval (Tampa-Hawthorne) is insufficient to account for the observed difference in fluorine accumulation. Alternatively, we may conclude that these bones have lain for long periods in distinct areas, perhaps not widely separate, but certainly percolated by ground waters of quite different fluorine content. Finally, the distribution of values in both worn and unworn bone suggests that such signs of wear as smoothed surfaces, rounded contours, etc., are neither exclusive nor quite reliable indications of transport.

References

1. OAKLEY, K. P. *Advancement of Sci.*, **6**, (24) (1950).
2. CARNOT, A. *Ann. Mines*, **3**, 155 (1893).
3. WILLARD, H. H., and WINTER, O. B. *Indust. Eng. Chem.* (Anal. ed.), **5**, 7 (1933).
4. WHITE, T. E. *Harvard Coll. Mus. Comp. Zool. Bull.*, **92**, (1) (1942).
5. OLSEN, R. Unpublished notes.

An Apparatus for the Simultaneous Production of Many Two-dimensional Paper Chromatograms

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Frequently in clinical laboratories or in the course of physiological, biochemical, or nutritional experiments it is an advantage to be able to run rapidly a large number of two-dimensional chromatograms of several specimens at

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