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.

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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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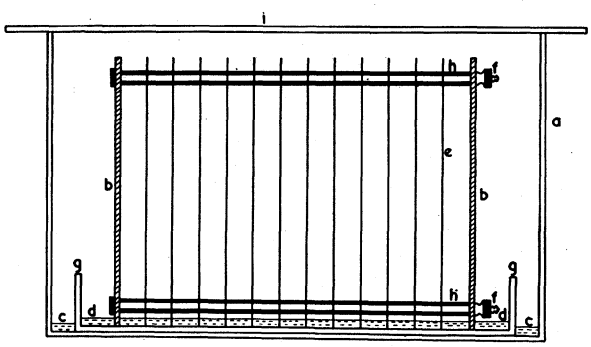

FIG. 1. Diagram of lateral view of apparatus. *a*, Tank, or box; *b*, end plates of frame; *c*, water; *d*, solvent mixture; *e*, paper; *f*, knurled nuts; *g*, dish; *h*, collar; *i*, glass lid.

the same time and under the same conditions. Using the large sheets of filter paper (18 in. × 22 in. or 30 in. × 30 in.) as described by Consden, Gordon, and Martin (*1*) and Dent (*2*), a great deal of space and equipment is necessary if one wishes to run more than 3 or 4 chromatograms daily, and the expense of solvents becomes serious.

We have found that a reasonable resolution of some 15 amino acids may be obtained on papers 20 cm square, using phenol and collidine-lutidine as solvents. The resolution is certainly adequate for the routine examination of urines, plasma ultrafiltrates, and cerebrospinal fluids. Twelve papers of this size are mounted parallel on a frame and are allowed to stand in a dish containing the first solvent, the whole being enclosed in a suitable box, or tank. The solvent creeps upward in the paper, and, when it has reached the top, the frame and papers are removed, dried, and placed in the second solvent so that it will travel in a direction at right angles to the first. The papers are then dried again, removed from the frame, and the spots developed visibly with ninhydrin in the usual way.

The frame (Fig. 1) consists of 2 duralumin plates 20 cm square and .3 cm thick; one plate is drilled and tapped with a 2BA thread in each corner, the centers of the holes being 1.5 cm from each edge of the plate. The second plate is drilled in line with the first with holes which will just clear a 2BA thread (4.8 mm). Into the holes in the first plate are screwed duralumin rods 28.2 cm long and .63 cm in diameter. These rods are threaded with a 2BA thread for .6 cm at the end, which is screwed into the plate, and for 1.6 cm at the other end; thus there are left 26 cm of unthreaded rod. These rods are fixed to the plate with knurled duralumin lock nuts .3 cm thick. The threaded part of the free ends of the rods passes through the holes in the second plate; these holes are not large enough to pass over the main part of the rod, and the plate may be fixed by knurled lock nuts 1.2 cm deep.

On each rod are 13 duralumin collars 2 cm long, 1 cm outside diameter, and .67 cm internal diameter. These slide easily on the rods and allow the papers to be kept apart. It is important that the collars fit loosely on the rods; otherwise, they will jam when the solvent is dried out.

The dish may be of duralumin, acid-resisting enamel, or porcelain. We have found that photographic developing dishes 35 cm × 28 cm are suitable, provided they have flat bottoms. The tank consists of an angle-iron framed glass aquarium 32 × 40 × 25 cm internal dimensions, covered with a well-fitting sheet of glass.

We have used both Whatman No. 1 and No. 4 filter papers, which may be obtained from the manufacturers in 20-cm squares. A hole is made in each corner of the paper to thread over the rods of the frame. A duralumin plate the same size as the end plates of the frame, with holes drilled in line, is used as a template in boring the holes. The holes in the template are large enough to pass a cork borer or other punch that will make a hole in the paper about .5 cm in diameter; this allows the paper to be a tight fit on the rods and to be held tightly stretched between them. With a template and a sharp cork borer it is possible to cut holes in 12 papers at a time. It is of great importance to align the papers correctly for boring so that when threaded on the frame their edges are parallel to and at the same level as those of the end plates. In this way a solvent front which travels parallel to the edge of the paper can be ensured.

We have used phenol (4 parts phenol, 1 part water) and collidine-lutidine (1 part each of collidine, lutidine, and water). These mixtures form one-phase systems with much less sensitivity to temperature changes than the mixtures usually described. Water is placed in the tank outside the solvent dish. These solvents do not corrode duralumin, nor does the metal appear to have any deleterious effect on the solvents or amino acids. No doubt other solvents could be used, but the resistance of the frame to corrosion by them should first be tested.

The mixture to be analyzed is applied in one corner of a paper at a point 3 cm from each edge, the spot being down to about 1 cm in diameter. We have found 5–25 μl urine, 50–125 μl plasma ultrafiltrate, and 625 μl cerebrospinal fluid to be satisfactory amounts in most cases. The papers are then threaded onto the frame, care being taken that the areas of paper to which the mixtures have been applied are all together at the same corner of the frame. Up to 12 papers may be put on the frame, each paper being separated from the next one, or the end plate, by a 2-cm collar on each rod. When less than 12 papers are being run, more than one collar may be used to separate them; in any case, the full number of 13 collars must be used on each rod so that there is no possibility of the papers moving or touching (Fig. 2).

The frame is then placed in the dish containing the first solvent (phenol), with the appropriate edge downward. The amount of solvent must be sufficient to allow all the papers to be completely wetted during the run and, at the same time, not so much that the area of paper holding the mixture to be analyzed is at all immersed in it. A few drops of ammonia are added to the water in the tank to keep the atmosphere alkaline. The lid is then put on.

When the solvent has reached the top of the papers, the frame is removed from the tank and placed in a stream of air to dry out the papers. When dry the frame is placed in the second solvent (collidine-lutidine) in another tank. Again the frame should be so placed that the solvent passes at once through the line of amino acids

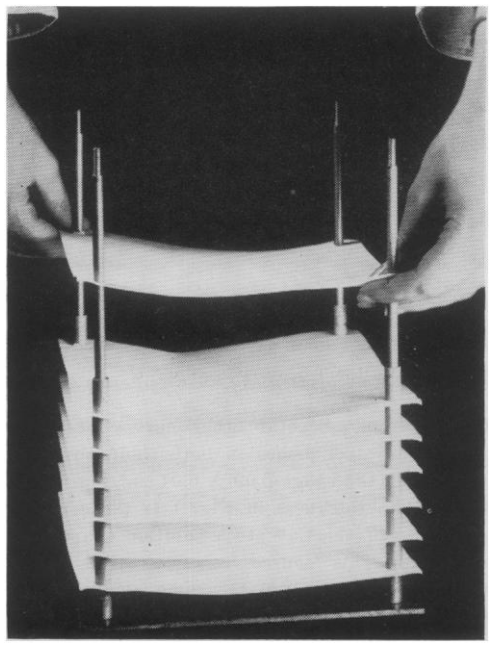

FIG. 2. Method of loading papers on frame.

formed during the phenol run and carries them along at right angles to their previous direction of movement.

When the collidine-lutidine solvent front reaches the top of the paper, there will be a brown line 3–4 cm behind it. No amino acids move in front of this brown line, and in order to increase their resolution in this solvent it may be made to overrun the chromatograms by laying sheets of filter paper horizontally in contact with their uppermost edges. These extra sheets of paper are held in place with thin sheets of glass, thus ensuring good contact. The run should be continued until the brown line almost reaches the top of the chromatograms.

When the second solvent has completed its travel, the frame is removed from the tank and the papers dried in a stream of air. They are then taken out of the frame, sprayed with ninhydrin, and developed by heating in the usual manner.

Using Whatman No. 1 paper, the run takes about 8 hr at 22° C in each direction. The frames may be put in the tanks at night and removed in the morning. Allowing the papers to stand in the tank for a few hours after the run is completed does not lead to any diminution in resolution. They may be dried during the day, and the second run carried out the next night. With 2 frames and 2 tanks it is possible to have 12 chromatograms completed each day, though the result of a particular test cannot be obtained for 48 hr. With Whatman No. 4 paper at 22° C, the run takes about 4 hr in each direction; thus a chromatogram may be completed in one day.

We have used this technique successfully for routine survey of large numbers of urines such as may be required in clinical work, for genetical investigations involving the analysis of urines of many members of the same family, and for physiological experiments involving the sampling of biological fluids over long periods of time. It would also be readily adaptable to semiquantitative work by running serial dilutions of the material to be studied, together with several dilutions of standard solutions on the same frame. Furthermore, there is no reason why the frame should not be lengthened considerably so that many more chromatograms may be run simultaneously.

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Self-Absorption and Backscattering of β Radiation[1]

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The complex nature of the absorption and scattering of β particles causes many difficulties in β counting, especially with low-energy β emitters such as C^{14}, S^{35}, and Ca^{45}, which are much used in biological work. It is often necessary to count sources of appreciable thickness for which self-absorption of the β radiation is significant. A further effect to be considered in the counting of thin sources is the backscattering of β radiations by the container or backing material used to support the source. This effect is strongly dependent on the atomic number (Z) of the backscatterer and is large for materials of high Z. Sources are often made "infinitely thick" for self-absorption, i.e., of thickness at least equal to the range of the β radiation, in order to avoid the large self-absorption corrections required for sources of intermediate thickness. However, for this case (as for any thickness greater than about 5% of the range of the β radiation) the effect of self-scattering (internal scattering) by the source must also be considered. From the experiments reported here it was found that self-scattering by the source increases with the average atomic number of the source material, as in the case of backscattering by the source holder.

An investigation of the above-mentioned effects was carried out with S^{35}, Ca^{45}, and P^{32} obtained from Oak Ridge. These nuclides are simple β emitters with maximum energies of 0.17 mev, 0.26 mev, and 1.7 mev, respectively. Counting was done with a Tracerlab end-window counter with window thickness of 1.5 mg/cm² and diameter of 2.8 cm. The counter was attached to a slotted aluminum shelf holder, which permitted counting of sources at various distances from the counter. The whole counter assembly was housed in an aluminum-lined lead shield. The coincidence loss correction for the counter was 0.6%/1,000 cpm.

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