STATE OF SOUTH DAKOTA
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SOUTH DAKOTA GEOLOGICAL SURVEY
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Circular 40

RECOVERING MICROVERTEBRATES WITH ACETIC ACID

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Vermillion, South Dakota
1970

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INTRODUCTION

Acid preparation of vertebrates has been known for some time (*e.g.*, Williams, 1953). Recently, Thaler (1966) mentions the use of acetic acid in the laboratory for the recovery of micro-mammals from carbonate rocks. He did not, however, go beyond stating that despite the simplicity of the use of acetic acid this method had not been previously employed on a large scale. It was the writer's privilege to learn and use this method in the *Laboratoire de Paleontologie, Faculte des Sciences, Universite de Montpellier* under the guidance of Louis Thaler and Jacques Michaux. Since then the method, modified as needed, has been successfully used at the South Dakota School of Mines and Technology. In addition to utilizing acetic acid in the laboratory, this method has been used in the field. Procedures for both are described here.

Acids other than acetic, such as hydrochloric, are not recommended. Hydrochloric, for example, has a tendency to destroy or etch the fossil. In addition, it is difficult to handle and in general not as safe to use as acetic. The safest and least offensive (to the nostrils) acid to use is formic acid. However, its high cost, about four times that of acetic, makes it impractical to use in large quantities.

PROCEDURE

The procedure for "L'attaque acide" as used by Thaler and his group is as follows:

1) Plastic tubs approximately 60 cm x 30 cm x 30 cm are used.

2) Next, place matrix in tub, adding enough water to cover.

3) Add glacial acetic acid in an amount to make an approximate five to ten percent solution. (A complete reaction takes at least 24 hours.)

4) Large lumps are removed and set aside to dry thoroughly before reprocessing. A heat lamp arrangement is used for increased drying efficiency. Drying is usually necessary as the saturated matrix does not react vigorously when returned directly into a fresh solution of acid.

5) The remaining material in the tub is passed through a coarse mesh (about 1.2 mm) wire screen under which a finer mesh (0.8 mm) wire screen is placed.

6) A spray with water is then passed over the upper screen.

7) All material not passing through the coarse screen is put with the large pieces for drying.

8) Material caught on the fine screen is washed in a sequence of tubs of water until no residue appears in the water.

9) The concentrate is then dried and is ready for picking. This is a general process and can easily be changed to fit particular situations.

PROCEDURAL MODIFICATIONS

In the laboratory of the Museum of Geology in Rapid City, essentially the same pattern has been used with the following modifications in materials. Heavy polyethylene tubs (Agile Division, Nalge Company, Rochester, New York) about 31 cm x 31 cm x 31 cm in size were used. Thin walled polyethylene wash tubs were tried for a time but were found to be difficult to handle when filled with water. They do make good tubs for rinsing. Instead of wire screen, polyethylene monofilament screen cloth (Kressilk Products, New York) with coarse screen cloth having 1.56 mm openings and fine screen cloth having .435 mm mesh opening was used. Polyethylene screen cloth has several advantages over galvanized wire screen which offset its initial high cost. It does not rust and is not affected by any chemicals except hydrocarbons. It does not stretch as does some metal screen such as aluminum. There are no sharp edges that abrade skin and it is easily cleaned. Our screens have been used almost daily for over two years with no loss of effectiveness, nor has it been necessary to halt the operation in order to make repairs either in the laboratory or field.

The first washing screens, about 46 cm x 46 cm in size, were made with ¾ inch x ¼ inch pine boards. Overlapping boards were nailed together with ordinary galvanized nails. Screen cloth was put into position and tied on with strong nylon thread. Any screen depth can be obtained this way. Ours range from 160 cm to 280 cm at the center. There are two disadvantages to making screens this way. First, the nails do rust. Secondly, the sewing of the cloth with nylon thread is time consuming. During an academic year one student processed one-half ton of matrix from one quarry and an equal amount from a second quarry. As used here, "processing" means reducing rock to concentrate by acid or other treatment for picking. One-half ton of matrix from a third quarry was processed in two months by two female students working eight hours per day. When processing was delayed, they picked concentrate.

For one-half of the year's operation only a few polyethylene tubs were in use. For the remainder of the year 25 heavy square tubs were used for acid breakdown. Inexpensive light-weight plastic tubs of the type first used, which can be purchased locally, were used for rinsing.

Indoor Processing

Indoor procedure for acid processing using polyethylene screen cloth:

1) Place matrix in tub; cover with water; add acetic acid.

2) When effervescence stops (sometimes up to three days), decant most of the liquid. Large sinks are more practical for this than small ones.

3) Place a fine mesh screen in a sink. Avoid allowing bottom of screen to rest in sink. A coarse mesh screen is fitted into it.

4) Remove large rocks from the tub by hand and set aside for drying.

5) Smaller pieces of rock are recovered by placing a ¼ inch mesh screen over the coarse plastic screen.

6) Pour the remaining contents from the tub over the screen assembly. Running water from a hose cleans out the tub.

7) Wash matrix on wire screen with running water; shake screen gently while washing. Put residue aside for drying.

8) Material on the coarse screen is rinsed with running water. If this concentrate is not too coarse to use as concentrate for picking, it is then washed in a series of water rinses and dried. If not satisfactory as a picking concentrate, it is set aside for drying and reprocessing.

9) Material on the fine screen is washed in running water until no visible sediment is passing through the screen. This is followed by a series of water rinses. Washing in this way has a double benefit. It generally removes all traces of calcium acetate which may effloresce on specimens if not removed, and it cleans specimens. Preparation thus becomes an incidental by-product of the recovery process.

Use of the coarse plastic screen is not absolutely necessary but it helps facilitate washing (Number 3, above). Material in a coarse screen should never be washed without a fine screen beneath it. Screens should not be overloaded. It makes cleansing more difficult and eventually takes more time and water.

Wet concentrate from screens has been dumped onto large pieces of corrugated cardboard for drying. These boards were salvaged from crates in which laboratory equipment and furniture was shipped. When the concentrate is dry, a board can be folded in the center and the concentrate funneled into suitable containers. Two or three pound coffee cans with clear polyethylene lids as containers for concentrate are convenient. Labels can be read through the lids, the cans store easily, and can be readily handled. Canvas can also be used for drying concentrate. Corrugated board has the advantage of absorbing water and increases the drying rate. The boards may also be placed on stands (of any kind) so that drying from underneath also takes place. There is essentially no breakage of specimens using this system.

One disadvantage to using many tubs simultaneously is that at the beginning of the processing period, the air is pungent with the aroma of acetic acid. The odor of large quantities of acetic acid in the laboratory can be alleviated by good ventilation. However, as efficient ventilation in the Museum of Geology laboratory was not possible, field processing was attempted.

Outdoor Processing

Basically, the method is the same as in the laboratory. In the field, however, the order of processing was modified as described below.

In the first year (1967) of this projected search for micro-mammals it was planned to collect matrix from quarries already known for their mega-mammal faunae and to search for new locations. The field party was fortunate in both instances. Very early in the field season, in two different formations, a concentration of tiny bones, teeth, and chips along with rock grains of similar size in layers from several centimeters to about 30 centimeters in thickness was discovered. The field party looked for similar layers wherever it prospected and was successful in a total of six localities of six attempted. Further experience prompts the writer to say that occurrences of such slightly coarser layers in fine sediments are probably more frequent than has been supposed.

For processing in the field the screens were re-designed. Since the screens were to be immersed in water, it was recommended that redwood boards be used because of redwood's resistance to water deterioration.

Care should be taken to use smooth boards as redwood splinters are known to be infective. Four 18 inch x 1¾ inch x ½ inch boards and four others 14 inches long were used for the frame of the screen. Two long slats with two short ones on one side were alternated by these sizes on the second (lower) side. The screen cloth was placed between the upper and lower sets more or less folded in the shape of a Maltese cross. Copper nails were used to nail the upper and lower sets of boards together. Although copper nails are expensive they do not rust in water. These screens are light in weight and do not take up much space when stored (fig. 1). For our type of washing and screening these screens are better suited than the heavy screen boxes the writer refers to as "Hibbard–McKenna boxes" (Hibbard, 1949; McKenna, 1962).

In choosing to process in the field, the South Dakota School of Mines and Technology field party has been fortunate to have its campsite along the Little White River which usually has a good flow of water. The camp was also within easy driving distance of several quarries.

Field Washing

The first step in field processing is to take sacked quarry matrix and sieve it through a ¼ inch wire mesh screen. The screen was placed over a canvas using two "Hibbard–McKenna" boxes for supports. The fine material was then washed in the river using the fine mesh

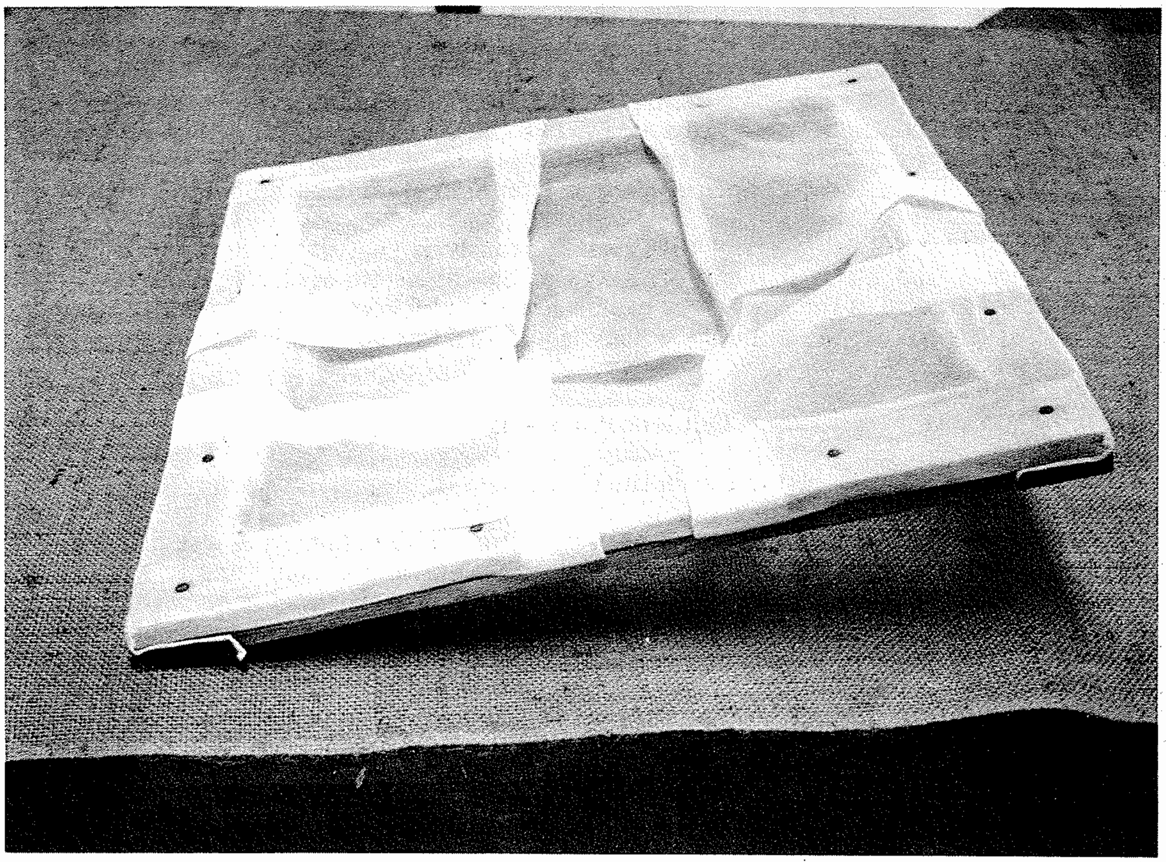

Figure 1. Fine mesh screen showing folds of screen cloth, overlapping of redwood boards, and copper nails.

screens. This step avoids unnecessary treatment with acid. While any system can be worked out, our most efficient method employing either two or three people and only 16 screens operated in the following way:

1) Two rows, each with eight worn out "Hibbard–McKenna" screen boxes were set up. Into each was placed one of our "Green–Martin" screens (fig. 2).

2) About one-third of a one pound coffee can of the dry screened material was placed on each screen.

3) One person carried a screen to the stream where a second person washed it. By the time the contents were cleansed, the screen handed back, another was given for washing. Washing involves a small amount of hand movement of the screen. It is a matter of judgment as to how much. Clay is not washed out without some movement. Although there is no proof, the writer thinks it is because of the high clay content in colloidal suspension of the waters in which the Museum of Geology field parties have done washing (Little Missouri River, Little White River). In fact, screen boxes left to soak in these waters may fill up with silt.

4) Washed screens were replaced into the old boxes but set in at an angle (fig. 2). The surface tension of the water on the fine mesh prevents rapid drainage. By placing the screens at an angle, water drips off. The screens were faced toward the sun as a start toward drying.

5) As soon as a screen stopped dripping water, a third person took it and dumped the contents onto a large canvas for drying.

6) The emptied screen was returned to a "Hibbard-McKenna" box and refilled with material to be washed.

In this way a continuous washing cycle using only a few screens was possible. It is also possible to operate with only two people but, of course, it takes longer to complete the work.

The second portion of field processing is similar to the laboratory method. It is mentioned here that dry screening and washing was not done in the laboratory because this phase had previously been done in the field.

Outdoor Acid Treatment

Out-of-doors it is necessary to use black polyethylene tubs according to the manufacturer, as white will deteriorate. One tub was used to carry water from the river to the other tubs. The acetic acid was in a 30 gallon drum. Acid was pumped out by squeeze bulb into a one gallon polyethylene container for convenient handling. Containers such as these are in one-half and one gallon sizes and many common "store-products" come in them. It is possible to get them, therefore, without cost. However, often these have a metal cap instead of polyethylene but with care even a container with a metal cap can be used. Small polyethylene pumps or siphons are more efficient than squeeze bulb pumps.

1) Up to 24 black polyethylene tubs are placed in two rows and rock added to each. Water is added to cover and then acid. It was found that usually three days were needed before the acid reaction halted. Whether this was a function of the matrix and acid or the open atmosphere is not known.

2) As in the laboratory, large pieces are removed and placed on canvas to dry.

3) In order to prevent any stream or soil contamination with either acetic acid or calcium

Figure 2. Fine mesh screens loaded with washed matrix; in old screen boxes. (These boxes are deep enough to allow placement of screens at an angle facing the sun to facilitate draining and drying.)

acetate pits are dug to a depth of a meter or so with a diameter sufficient to hold the frame of a screen. (Calcium acetate is generally non-toxic. The lethal dose in rats is 4.28 grains per kilogram of weight. Both it and acetic acid will be degraded in soil.)

4) A fine mesh screen is placed over the opening and over it a ¼ inch wire mesh screen.

5) Contents of an acid tub are poured on and washed with water.

6) Residue on the wire screen is set aside for drying.

7) The fine screen is then placed in a "Hibbard–McKenna" box.

8) When a sufficient number are ready, they are washed in the stream as previously described for dry screened matrix.

Circumstances of number of personnel, weather, etc., do dictate modification of the general washing cycle.

Large metal containers of various types have been used to store dried concentrate. Old ammunition cases may be purchased in surplus stores. Also obtainable from restaurants and dairies, without charge, are large cans (with or without handles) in which foodstuffs have been stored.

Other uses for the fine mesh polyethylene screen cloth can be found. For example, if a large specimen in rock such as a skull is to be prepared by acid, the specimen can be wrapped in it (fig. 3). The specimen is then immersed in the acid solution. The advantage of doing it this way rather than immersing the specimen without wrapping it is that if pieces fall off, they are easily recovered. Further, when the reaction ceases, the specimen can be washed in running water still within its wrapping. Placing the specimen in a suitable container of water and allowing gently running water to wash over the specimen effectively removes acid and acid reaction by-products.

Similarly, if a small specimen such as a tooth still has a quantity of matrix on it, it can be placed in a small tray made by folding the polyethylene cloth (fig. 4) and the tray placed in a dish of acid. The reaction may even be observed under a binocular microscope. When ready for washing, the specimen is left in the tray and placed in lightly running water as previously described. In the laboratory at Montpellier treatment to get small particles of matrix out of tiny fossettes, etc., utilized hydrogen peroxide (H_2O_2). However, there is a tendency for H_2O_2 to burn the skin and it causes the hair to fall out of the small brushes usually used to maneuver small teeth and bones. Other objections to H_2O_2 are that the bubbles may be large enough to break cracked specimens. But more, concentrated H_2O_2 is potentially explosive and special care is needed in removing it from a carboy. It is then diluted for use.

Trays may be made of the heavier polyethylene cloth by folding and sewing the folds with nylon thread (fig. 5). We have used scraps left over from making the large screens for "bags" and "trays" of varying sizes as needed. If simply folding the cloth does not appeal to the user, application of heat to the folds will fuse them.

The efficiency of the fine mesh screen for washing is attested to by the recovery of small heteromyid teeth measuring 0.5 mm by 0.5 mm. Certainly, the smallest teeth are lost through ordinary window screen. Teeth of extremely small size may not be seen with the naked eye as picking is generally practiced in the field. Although an experienced individual may be quite efficient, saved "field picked matrix" examined with a low power binocular microscope in the laboratory has uncovered additional specimens.

ACKNOWLEDGEMENTS

I wish to acknowledge my thanks to James Soiset who constructed the first of the polyethylene screens and to James E. Martin for making the second set. Mr. Martin also helped in working out the process in the laboratory and in the field. Special thanks are

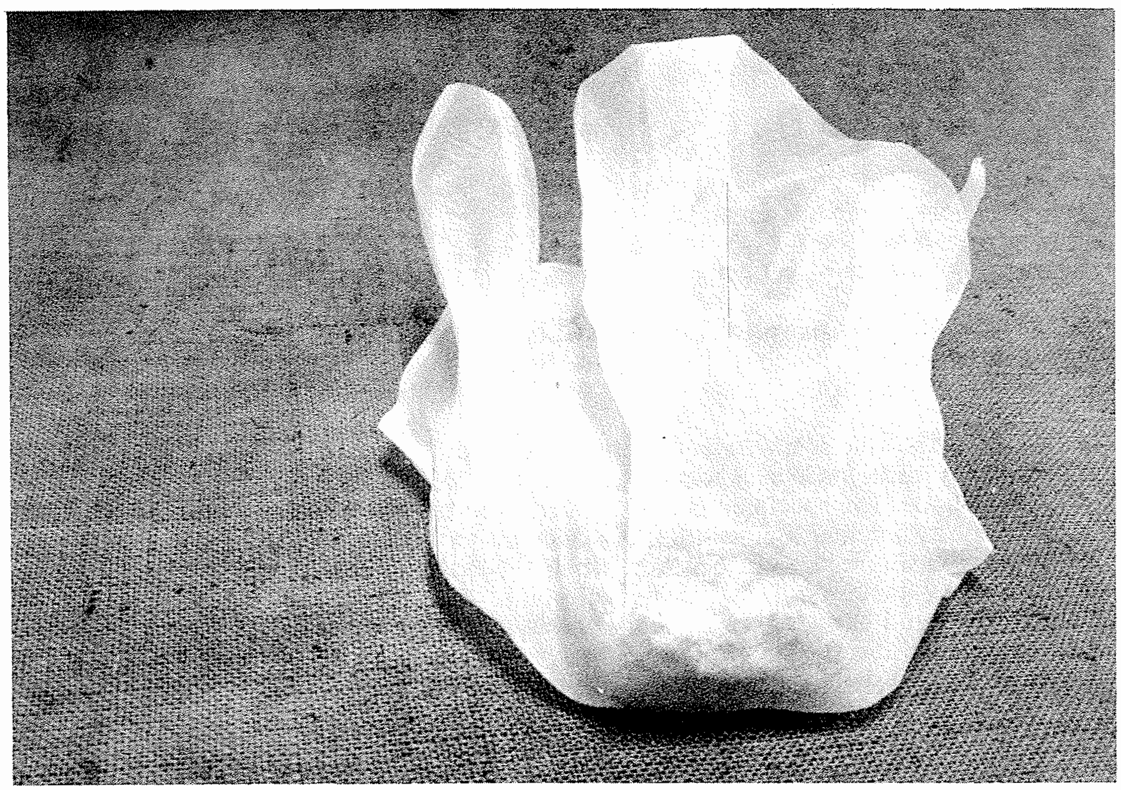

Figure 3. *Merycoidodon* skull fragment in fine mesh "bag." (Pieces of bone that might fall off are retained in the bag.)

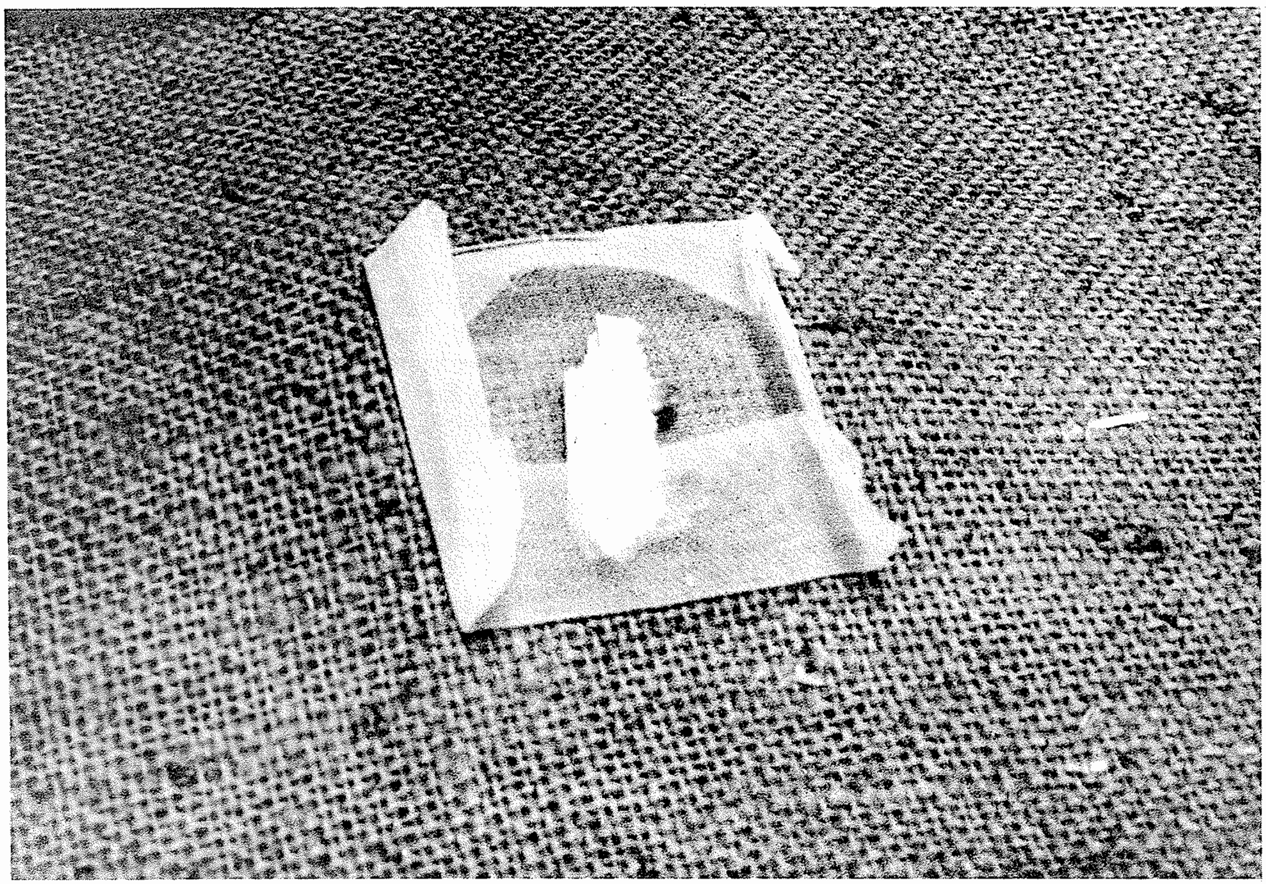

Figure 4. Small fine mesh screen tray, 6 cm x 6 cm with jaw fragment. (Smaller specimens can be completely but loosely wrapped.)

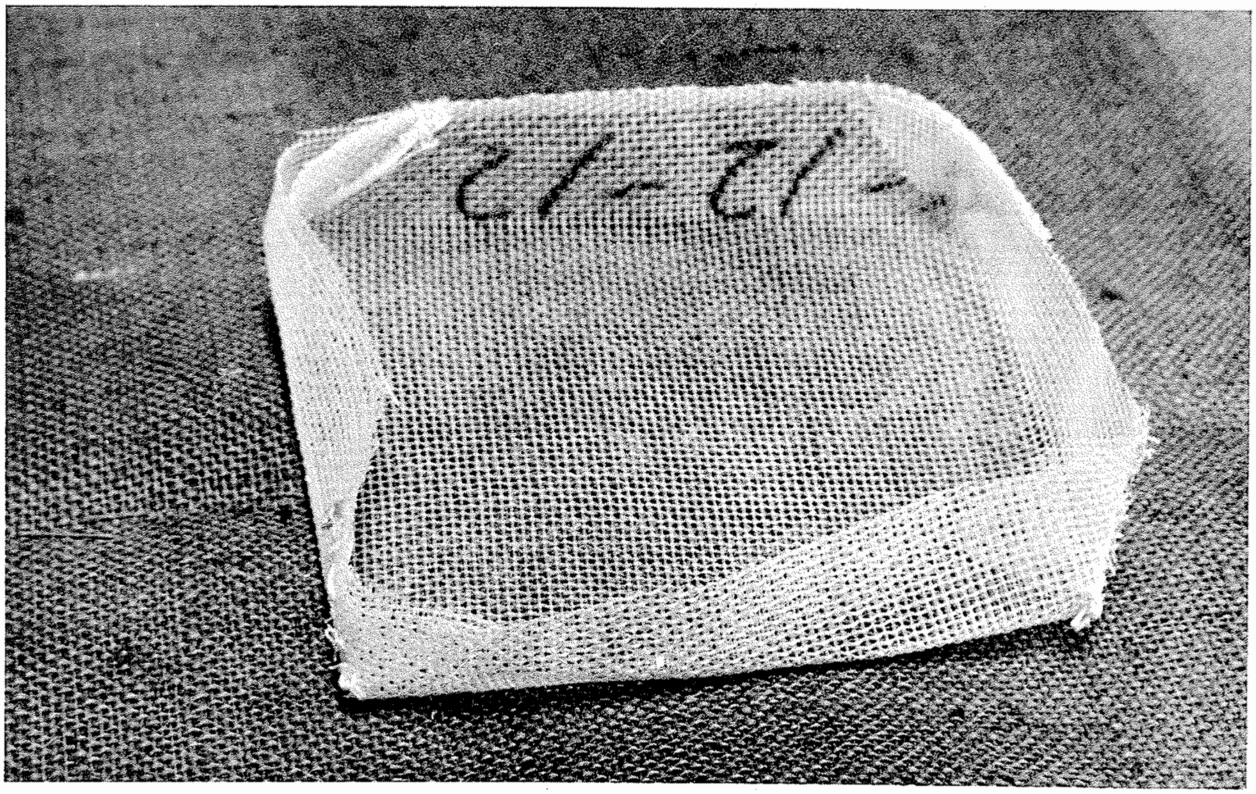

Figure 5. Large mesh screen tray, 16 cm x 16 cm; corners sewn with nylon thread.

extended to Mr. and Mrs. D. C. Rice and Alan Rice, not only for allowing us to camp on their ranch but also for providing us with material so that we could fence off an area along the Little White River to keep cattle away from our washing and screening equipment. Many thanks also to Louis Thaler and his associates for introducing me to the mass treatment of carbonate matrix with acid for the recovery of small vertebrates. My work in Montpellier was partially supported by Grant No. 3886, Penrose Fund of the American Philosophical Society.

The photograph presented as figure 1 was taken by the writer. The remainder were taken by J. C. Harksen.

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