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Recovery of Parasite Remains From Coprolites and Latrines: Aspects of Paleoparasitological Technique

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Recovery of Parasite Remains From Coprolites and Latrines: Aspects of Paleoparasitological Technique

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Introduction

Paleoparasitology has been the focus of intensifying research during the past decade (ARAUJO et al. 1985; CONFALONIERI et al. 1981,1985; FRY 1985; FOUANT 1981; GOOCH 1983; HERRMANN 1986, 1987; JONES 1983, 1985, 1986; KLIKS 1983; REINHARD 1985a, 1985b, 1985c; REINHARD et al. 1985, 1986, 1987; STIGER 1977; HORNE 1985 for review of New World finds through 1984). Paleoparasitological research involves the examination of colon contents, coprolites (desiccated feces), and latrine soils. Occasionally, infection is evidenced by osseous involvment, especially with echinococcosis (ORTNER and PUTSCHAR 1981: 229-233; WEISS and MØLLER-CHRISTENSEN 1971; WILLIAMS 1985).

Although many reports of parasitological finds are now available, the techniques utilized by paleoparasitologists and discussions of the preservation of prehistoric helminths (roundworms, tapeworms, thorny-headed worms, and flukes) have never been summarized. Consequently individuals who are initiating paleoparasitological work are frustrated at the onset by lack of information regarding effective techniques. Presented below is a discussion of helminth preservation in archaeological contexts and a summary of techniques applied by paleoparasitologists to latrine soils and coprolites (desiccated or mineralized feces). Some techniques are evaluated experimentally. Citations to clinical techniques used in paleoparasitological research are provided. Techniques innovated by the authors and other paleoparasitologists are described.

Helminth Preservation

The main evidence of prehistoric helminth infection is the find of parasitic worm eggs. Although these are preserved in a variety of environments, preservation seems to be best in moist anaerobic environments or desiccating environments. Latrines readily provide for the former conditions while caves in arid regions provide the latter.

Desiccation allows the preservation of coprolites (BRYANT and WILLAIMS-DEAN 1975) and mummies (ALLISON et al. 1974; FOUANT 1981). Nematode larvae, as well as eggs, are found in desiccated remains (ARAUJO et al. 1981; FERREIRA et al. 1980, 1983a; HALL 1972; REINHARD 1985a, 1985b; SAMUELS 1965). Analyses of coprolites and colon contents of mummies are most frequently reported from the deserts of the southwestern United States (FRY 1977,1980; FRY and HALL 1969, 1975; MOORE et al. 1974; REINHARD 1985a, 1985b, 1985c; REINHARD et al. 1985; SAMUELS 1965; STIGER 1977L central and northeastern Brazil (ARAUJO et al. 1980, 1981; CONFALONIERI et al. 1981; FERREIRA et al. 1980, 1983a, 1983bL and Peru (CALLEN and CAMERON 1960; PATRUCCO et al. 1983; WEIR and YONAVIA 1985). Cold, dry environments also result in the preservation of helminth remains in mummies (PIZZI and SCHENONE 1954).

Latrine soils have most frequently been described from Europe (GOOCH 1983; HERRMANN 1986; JONES 1983,1985; PIKE 1967, 1975; TAYLOR 1955) and a few studies have been done in the New

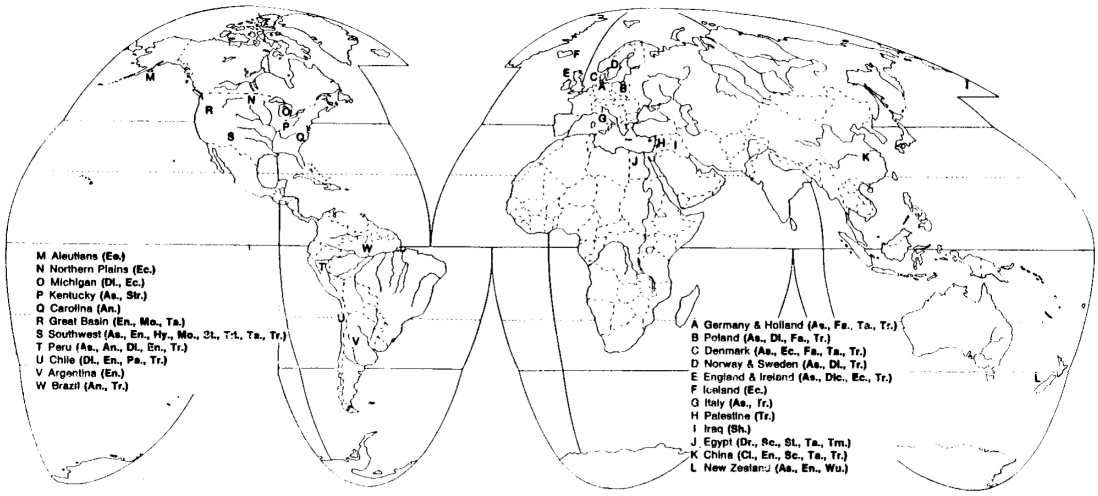


Figure 1: Analyses of fecal debris from archaeological sites resulted in the find of many cases of parasitism. The finds to date are mapped below. For the Americas, the finds noted as TAENIA are considered only to be in the family Taeniidae. The identification to genus level is not yet established for these finds. An. = *Ancylostoma*, As. = *Ascaris*, Di. = *Diphylloborthrium*, Cl. = *Clonorchis*, Dic. = *Dicrocoelium*, Dr. = *Dracunculus*, Ec. = *Echinococcus*, En. = *Enterobius*, Fa. = *Fasciola*, Hy. = *Hymenolepis*, Mo. = *Moniliformes*, Pa. = *Paragonismus*, Sc. & Sh. = *Schistosoma*, St. = *Strongyloides*, Ta. = *Taenia*, Tr. = *Trichuris*, Tri. = *Trichostrongylus*, Trn. = *Trichinella*, Wu. = *Wucheria*. For Germany, also the presence of Dic., Di., and En. could be proven.

World (HEVLY et al. 1979; REINHARD et al. 1986). Mummies, and their associated helminths, have been preserved by freezing (ZIMMERMAN and SMITH 1975). In addition, colon contents of mummies from peat bogs in England and Denmark have revealed cases of helminth parasitism (FISCHER 1980; JONES 1986). These results of these analyses have provided substantial evidence of ancient parasitism (Figure 1).

Preservation of helminth eggs found in latrine soils is generally good. Often, even delicate eggs remain uncollapsed. Opercula and polar plugs of certain species are usually intact. Opercula are common among trematode (fluke) and certain cestode (tapeworm) taxa, and polar plugs are typical of trichurid and capillarid nematodes. The embroyos of eggs in archaeological latrines are sometimes preserved to some degree. Such preservation was reported by PIKE (1967) from medieval deposits in York, United Kingdom and from prehistoric Indian latrines at Elden Pueblo, Arizona studied by HEVLY and ANDERSON (HEVLY et al. 1979). The York deposits revealed eggs of *Trichuris* sp., *Ascaris* sp., and *Dicrocoelium dendriiicum*, The Elden Pueblo soils contained the eggs of *T. trichiura*, *A. lumbricoides*, *Enterobius vermicularis*, as well as taeniid and hyrnenolepidid cestodes. One problem that is unique to fecal deposits in open sites is the washing of parasite eggs out of soil by water activity (CONFALONIERI, FERREIRA, ARAUJO, unpublished observation).

Unusually poor preservation of eggs in latrine soils is characteristic of the latrines excavated at Queen Anne Square in Providence, Rhode Island (REINHARD et al. 1986). The most common eggs found in the Queen Anne Square soils were those of *A. lumbricoides* and *T. trichiura*. Especially poor preservation was exhibited by the trichurid eggs. Some of the eggs were enveloped in fungal mycelia. The polar plugs of the eggs were usually gone and the embryos were rarely present. A few eggs were chracked or shattered. It was not uncommon to find fragmented eggs.

It is probable that two elements of decomposition were at play in the Queen Anne Square latrines. Fungal organisms were apparently involved in the decomposition of the eggs, perhaps by first digesting the polar plugs and then the embryos. Secondly, fragmentation of the eggs possibly resulted

from mechanical stress such as freezingthawing episodes. It is interesting that the *A. lumbricoides* eggs were generally in good preservation with embryos intact. This suggests that differential preservation of eggs should be considered in paleoparasitological examination of latrine soils, as suggested by PIKE (1975).

HERRMANN notes that only very few *Taenia* sp. egg have yet been recovered from Medieval latrine deposits from West German sites. It is assumed that either the eggs are too delicate to preserve there, and/or the inspection of Medieval meats in that area was especially effective.

With respect to West German latrine soils, no significant relation between pH level and egg count has been found to date. The soil samples from latrine deposits examined by HERRMANN ranged in pH value from 5.8 to 9.0. All of these samples contained various amounts of eggs. No statistical relation between egg per gram counts and pH were demonstrated (Figure 2).

Coprolites provide excellent evidence of helminth parasitism. However, the desiccation process can be damaging to certain helminth eggs. In the authors' experience, collapsing, wrinkling and cracking of helminth eggs are the main problems evident in eggs recovered from coprolites. However, in experimental desiccation of *T. trichuria* eggs, CONFALONIERI et al. (1985: 9) note that only some of 100 eggs examined exhibited "gross alterations such as vacuolization of the embryonic mass, discoloration, corrugation of the shell outline, and also lateral deformities resulting in the loss of symetry in

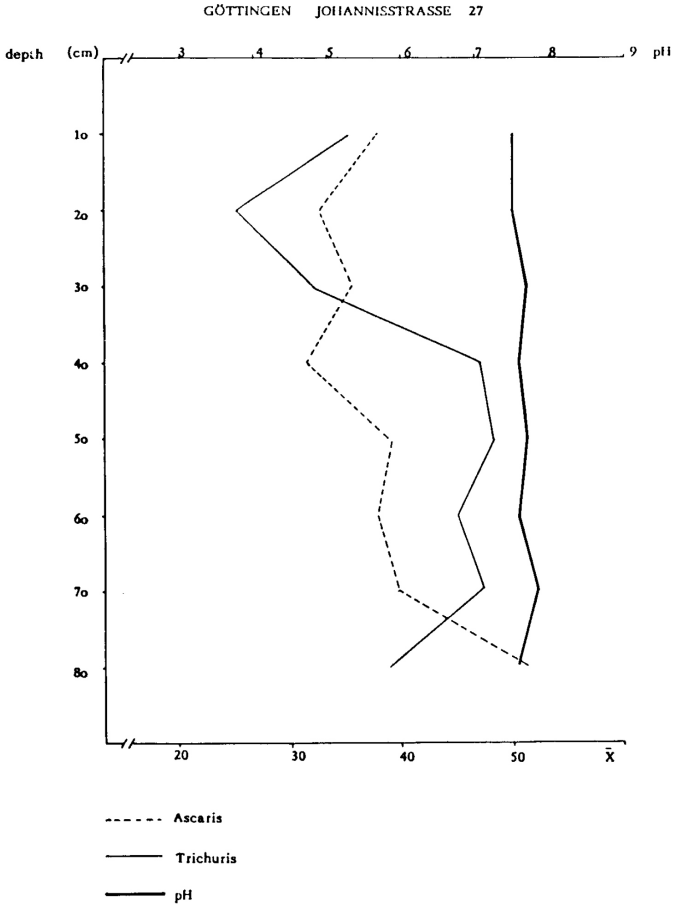
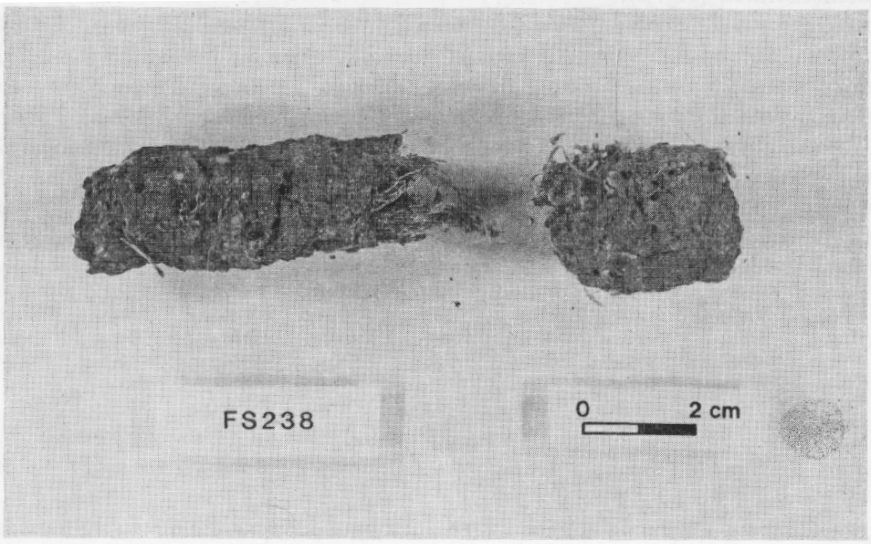


Fig. 2: Egg counts and pH value for levels from a medieval latrine deposit from Göttingen, West Germany (egg counts to be multiplied by 100 to determine the number of eggs per gram of soil).

the structure." The majority of the eggs were well preserved and the ability of the researchers to identify the eggs of this species was not impaired.

Rapid desiccation results in well preserved coprolites (Figure 3). In general, preservation of helminth eggs in coprolites is excellent. This is best reflected by the frequent recovery of delicate pinworm eggs from coprolites (ARAUJO et al. 1985; FRY 1977, 1980; FRY and HALL 1986; FRY and MOORE 1969; Hevly et al. 1979; REINHARD 1985a; REINHARD et al. 1985; SAMUELS 1965; STIGER 1977). Usually, the larvae inside helminth eggs in coprolites are preserved. We have, however, found cases in which the larvae and eggs are relatively poorly preserved. These cases come from open Indian



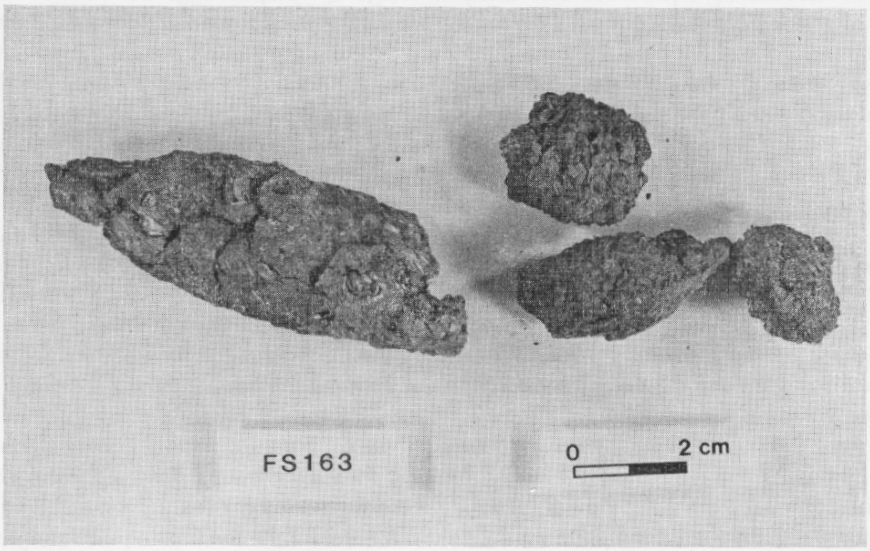


Fig. 3: Coprolites from Turkey Pen Cave from the Grand Gulch of Utah, USA showing the excellent preservation of ancient feces by desiccation. These examples date to the third centuray A. D.

sites in which feces have decomposed to a certain degree before complete desiccation. In such cases, coprolites invariably contain remains of scatophagous beetles, mites, nematodes, and/or fungi.

Desiccation is usually a rapid process. Helminth species that normally hatch out of their eggs and

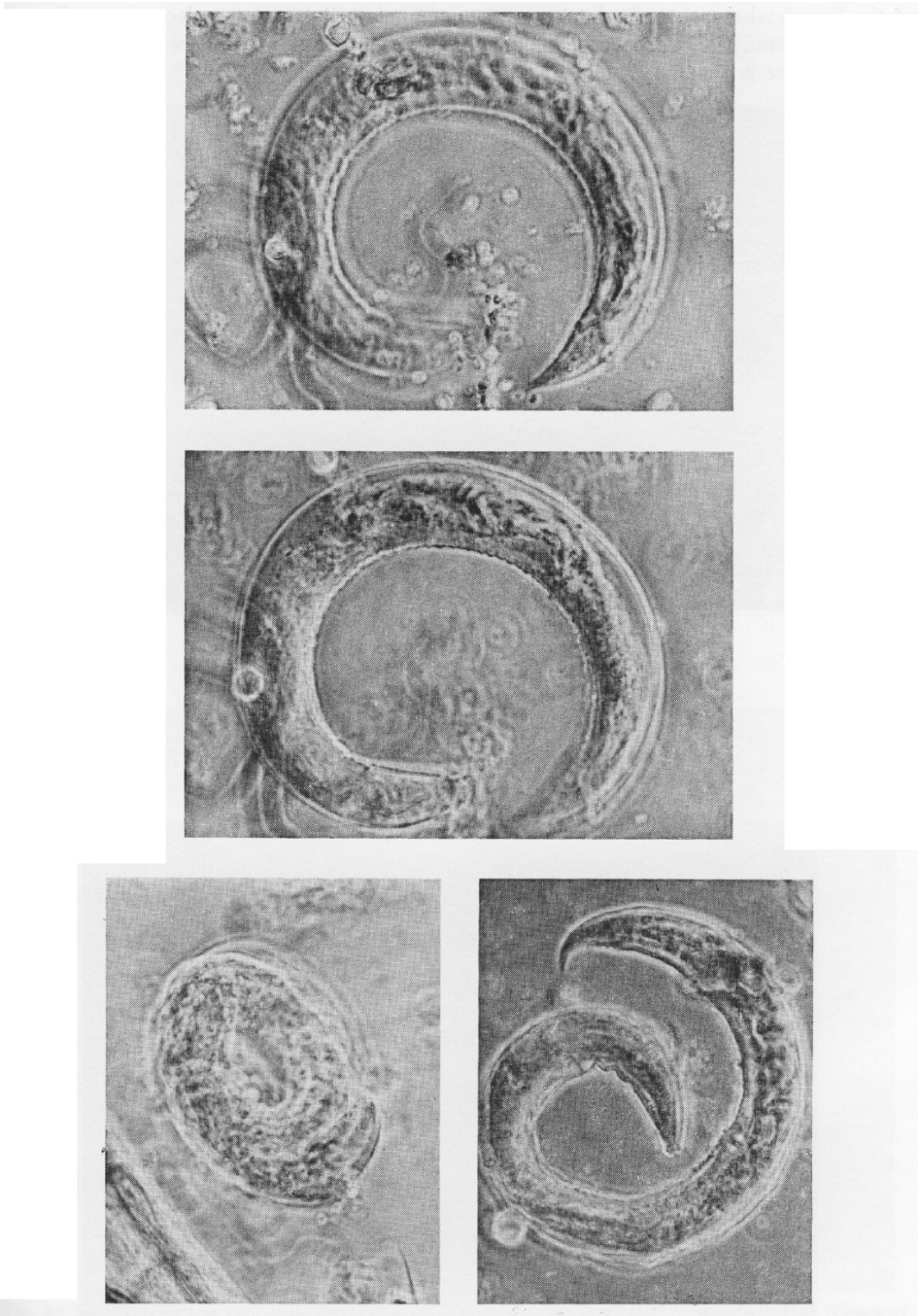


Fig. 4: Rapid desiccation of feces leads to the preservation of nematode larvae. These larvae, probably of the genus Strongyloides, were recovered from coprolites dating to A. D. 1100—1200 from Antelope House in Canyon de Chelly National Monument, Arizona in the USA

leave feces are trapped by drying (Figure 4). This results in the find of hookworm (FERREIRA et al. 1980, ARAUJO et al. 1981), *Trichostrongylus* (REINHARD 1985a), and *Strongyloides* (HALL 1972; REINHARD 1985a, 1985c). Once these nematode larvae hatch from their eggs (or are defecated in the case of *Strongyloides*) they initiate a free-living stage in their life cycle. Desiccation results in the excellent preservation of the larvae whereas, in the latrine environment, free-living stages decompose.

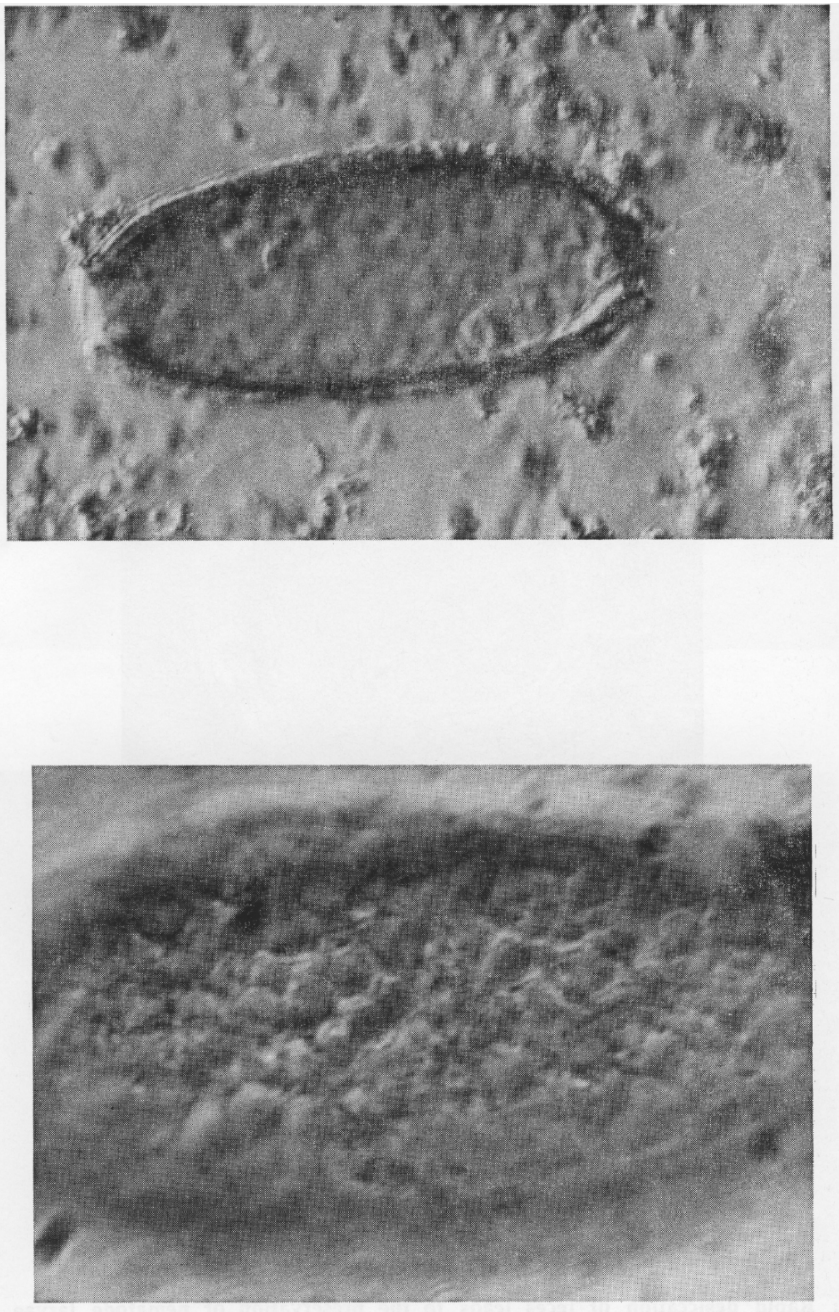


Fig. 5: In some cases, the preservation of the egg morphology is excellent but there is poor or no preservation of the larvae within the eggs as seen in this capillarid egg from the Windover Site, Florida, USA. The egg is approximately 6,000 years old.

Consequently the study of coprolites is an excellent avenue for the study of helminth diversity in a host population.

In unusual circumstances, excellent preservation of helminths in coprolites can not be expected. For

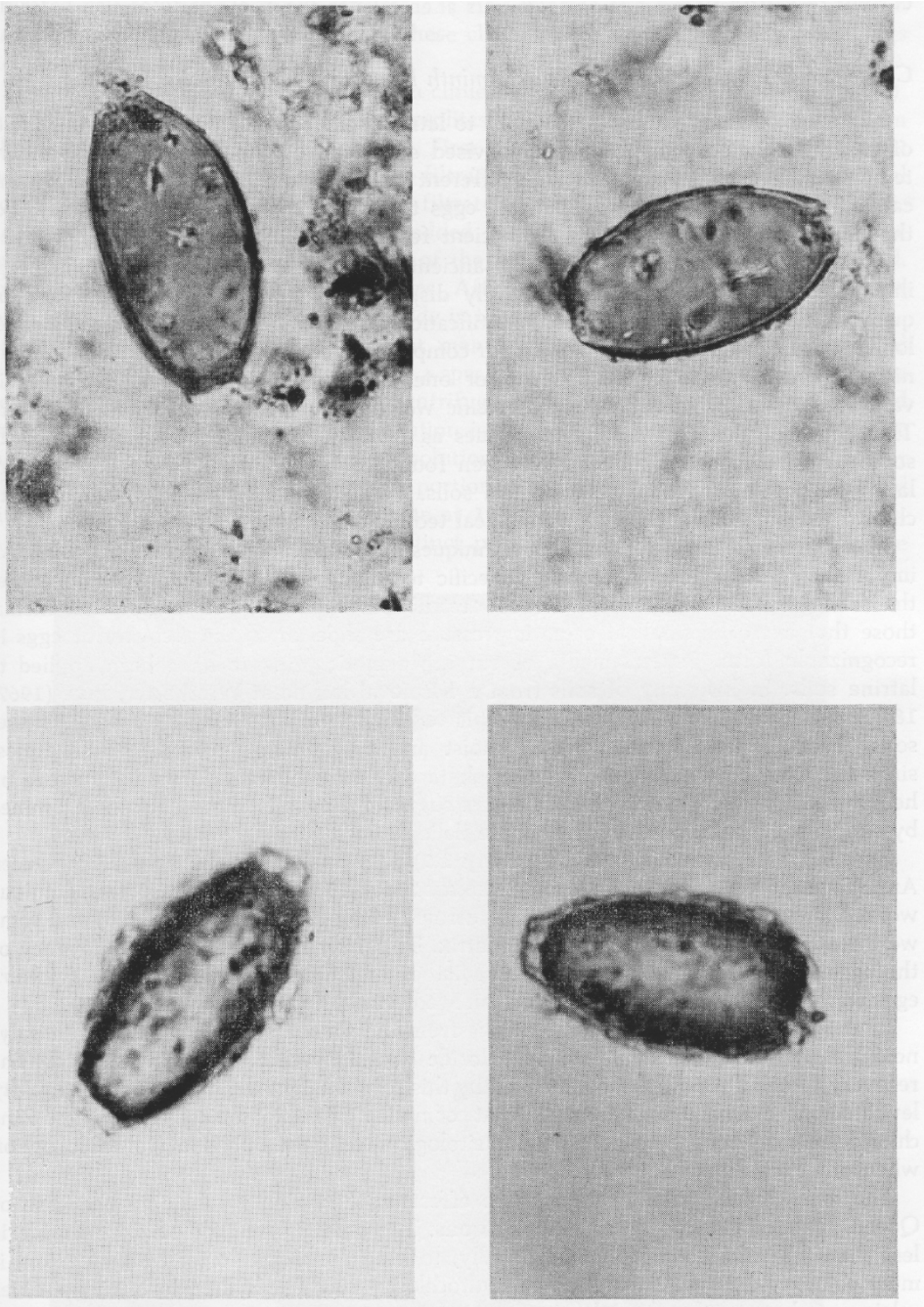


Fig. 6: TRICHURIS eggs recovered from latrine soils from Queen Anne Square, Newport, Rhode Island, USA. The upper eggs were extracted by palynological processing. The lower eggs were found by sodium chloride flotation. Note the encrustation of the lower eggs as well as breakage of the egg on the lower right. The latrines date to the period of the American Revolution.

example, a recent examination of alligator feces found in a Florida bog dating to 6,000-7,000 years B. P. revealed two species of *Capillaria* and three trematode species. Although the eggs shells are excellently preserved, the larvae within the eggs are completely decomposed (Figure 5). This is true even for the capillaricl eggs which have intact polar plugs and trematode eggs with intact opercula (REINHARD, current research).

Concentration and Identification of Helminth Eggs from Latrine Soils

There are several techniques to apply to latrine soils. One can select from a variety of clinical techniques or techniques devised specifically by paleoparasitologists. The techniques have been developed with different purposes in mind. The purpose of clinical techniques is simply to concentrate eggs to prove their presence more easily. For the clinician, only a few eggs are sufficient for a clinical diagnosis.

The purpose of techniques applied to ancient latrine deposits is different. Since eggs in archaeological contexts are extensively dispersed by a number of agents, a more quantitative approach is required. Quantification also provides a basis for an epidemiological approach and provides a basis for comparative studies. In general, clinical techniques have found to be insufficient for one primary reason: since eggs appear in various states of preservation, their specific weight is influenced in various directions. Thus the fruitful use of clinical techniques as a basis for quantification is disabled. In some cases, clinical techniques have been found to be completely ineffective in isolating parasite eggs from archaeological soils. With this caveat, we will review both clinical techniques and paleoparasitological techniques used in various studies.

In some cases, clinical floatation techniques are applicable to latrine deposits. An important consideration in choosing specific techniques is the selection of procedures that cause minimal egg distortion or destruction. With respect to flotation media, those that exert the smallest osmotic pressure are superior in the recovery of eggs in recognizable form. Several media of varying osmotic pressure have been applied to latrine soils. In his study of soils from a Medieval latrine at Winchester, PIKE (1967: 187-188) used zinc sulphate flotation. This technique worked well with the Winchester soils "because the material was still moist and apparently had remained so almost since the time of deposition." Zinc sulphate exerts considerable osmotic pressure on helminth eggs. Recognizing this fact, PIKE suggests that soils should also be examined by simple sedimentation to recover more delicate eggs.

Zinc sulphate solution (specific gravity 1.2) was applied to the soils from Queen Anne Square, Newport, Rhode Island (REINHARD et al. 1986). In this case, the technique worked less well. Some of the eggs were surrounded by fungal growth (Figure 6) and some were trapped in a calcium carbonate matrix. This may have limited the buoyancy of the eggs in the zinc sulphate medium. Microorganism decomposition of *T. trichiura* eggs were noted in the analysis of german Medieval latrine soils as well (Figure 7).

In an effort to levitate identifiable eggs from the Queen Anne Square soils, a palynological flotation medium of zinc chloride at 1.5 specific gravity was tried. The recovery of helminth eggs was enhanced by this solution, but the higher specific gravity levitated large amounts of detritus that complicated microscopic examination. Zinc chloride is not frequently used in parasitological research but is a workable option when other techniques are not effective.

The zinc chloride medium was more effective than zinc sulphate in the case of Queen Anne square for at least two reasons. The osmotic pressure of zinc chloride is less than that of zinc phosphate. Secondly, to keep the zinc chloride in suspension, it must be mixed in a 10% solution of hydrochloric acid. The hydrochloric acid disolves calcium carbonate, thereby releasing some eggs.

Another flotation solution that was effective in levitating eggs from the Queen Anne Square latrine soils was sodium chloride (specific gravity 1.2) (REINHARD et al. 1986). This solution exerts the least osmotic pressure on helminth eggs of the three solutions applied to the Queen Anne Square soils. It

was effective in levitating well preserved and damaged eggs, some of which were covered with mycelia or carbonates. This is a technique of choice (REINHARD) since it is easily obtainable by making a saturated salt solution. It is not caustic as is zinc chloride and produces less distortion than zinc sulphate. However, none of these clinical techniques concentrated the eggs enough to allow for quantification.

Zinc phosphate flotation is described in clinical texts (see MELVIN and BROOKE 1974). A modified procedure for use with coprolites is presented by FRY (1977). Sodium chloride is prepared in the following way. First the soil sample is screened through 0.9 and then 0.3 millimeter mesh screen. The filtrate is concentrated either by gravitational sedimentation or centrifugation. The soil filtrate is transferred to a shell vial or centrifuge tube and the flotation medium is added such that the fluid 'completely fills the container. A coverglass is placed on top of the preparation, in contact with the fluid. The preparation then stands for 5 minutes. After this time, the coverglass is removed and placed on a microscope slide. The slide is then examined.

Zinc chloride is prepared in a different manner. Zinc chloride crystals are disolved in a 10% hydrochloric acid solution until a specific gravity of 1.3 is obtained. The soils are screened as above and the filtrate centrifuged. The supernatant is poured off and zinc chloride solution is added. The solution is stirred to ensure that the soil filtrate and flotation solution are mixed. The solution is centrifuged at 1,900 r. p. m. for ½ hour. After centrifugation, the upper portion of the supernatant is removed, diluted with water, and centrifuged for 1 minute at 1,200 r. p. m. This portion of the supernatant some

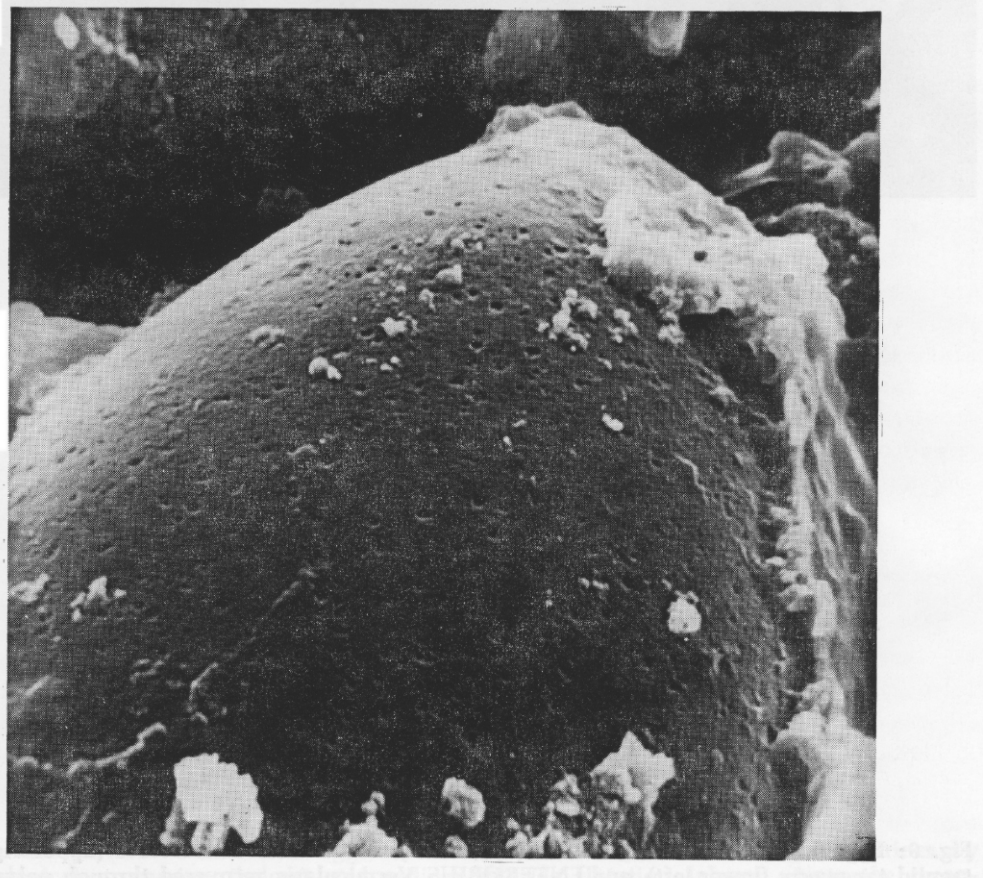


Fig. 7: A TRICHURIS egg from a medieval latrine deposit from West Germany showing punctate damage probably caused by microorganisms (x 4,700).

times is in the form of a distinct plug of solid material at the top of the solution. More frequently it appears as a thin area of fluid containing small bits of debris. When the second centrifugation is complete, the supernatant is poured off. A small amount of the sediment is mounted in glycerol for microscopic examination.

Other flotation media have been used. GOOCH (1983) reports that sucrose flotation can also be utilized.

The fortuitous find of helminth eggs in pollen preparations from Mt. Elden Pueblo (HEVLY er al. 1979; Richard H. HEVLY, personal communication) suggested that palynological techniques might be utilized in recovering helminth eggs from latrine soils (Figure 8). Palynological extraction techniques were applied to soils from Queen Anne Square with good results. The process involves treatment of soil samples in sequential baths of concentrated hydrochloric acid, water, hot 40% hydrofluoric acid, water, lacial acetic acid, hot acetolysis solution, glacial acetic acid, water, potassium hydroxide, and

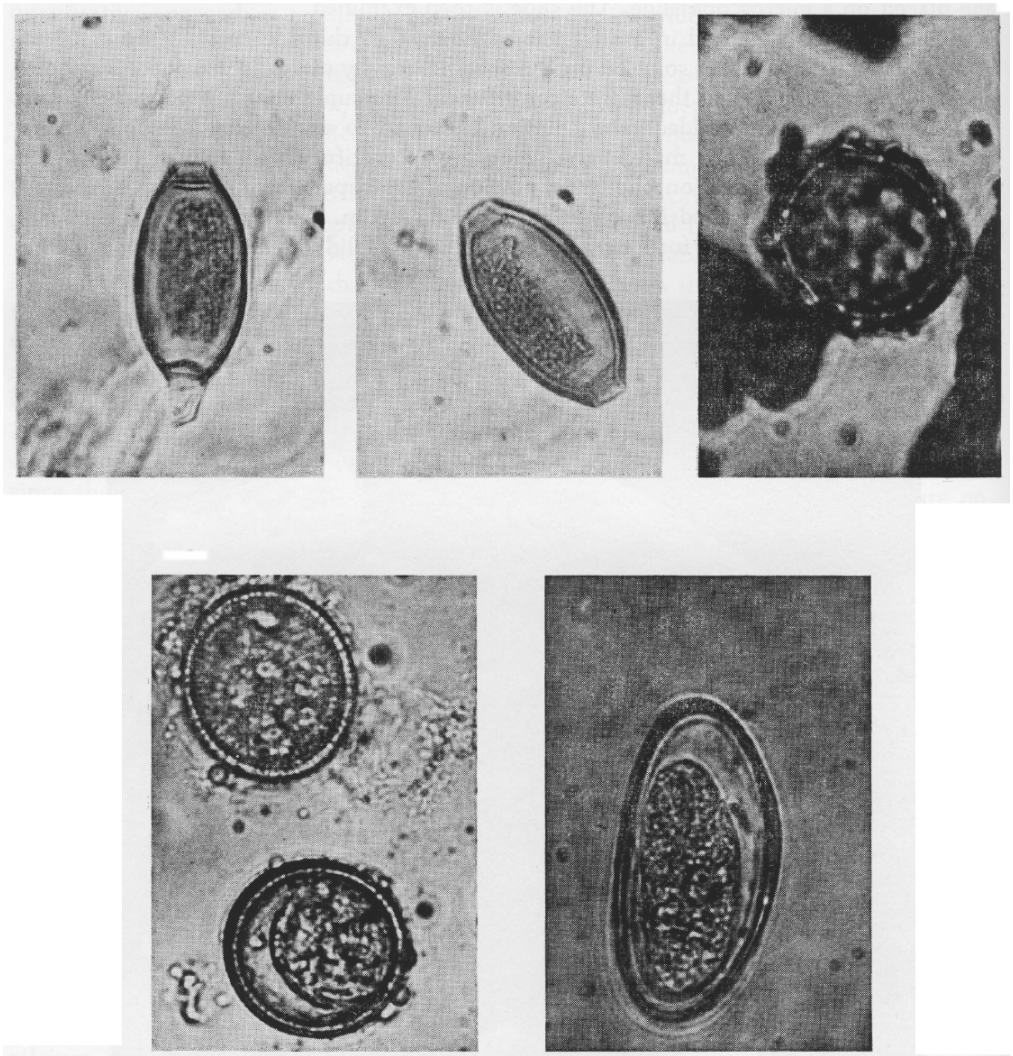


Fig. 8: Eggs of TRICHURIS Trichiura (upper left), ASCARIS Lumbricoides (upper right), a taeniid tapeworm (lower left), and ENTEROBIUS Vermicularis recovered through palynological processing of latrine soils from Mt. Elden Pueblo near Flagstaff, Arizona, USA. The eggs date to about A. D. 1250.

water. Through this method, sufficient numbers of helminth eggs were recovered from the Queen Anne Square deposits to allow quantification and comparison between latrines (REINHARD et al. 1986).

To evaluate the durability of helminth eggs during this process, a modern fecal sample containing eggs of *Ascaris lumbricoides*, *Clonorchis sinensis*, *Schistosoma japonicum*, and *Taenia pisiformis* was treated with palynological processing procedures. After each stage in the process, a sample of the soil was examined. Between 500 and 1,000 eggs were counted for each examination and the ratios of the different species calculated. No evidence in reduction of overall number of eggs, nor change in the ratios was

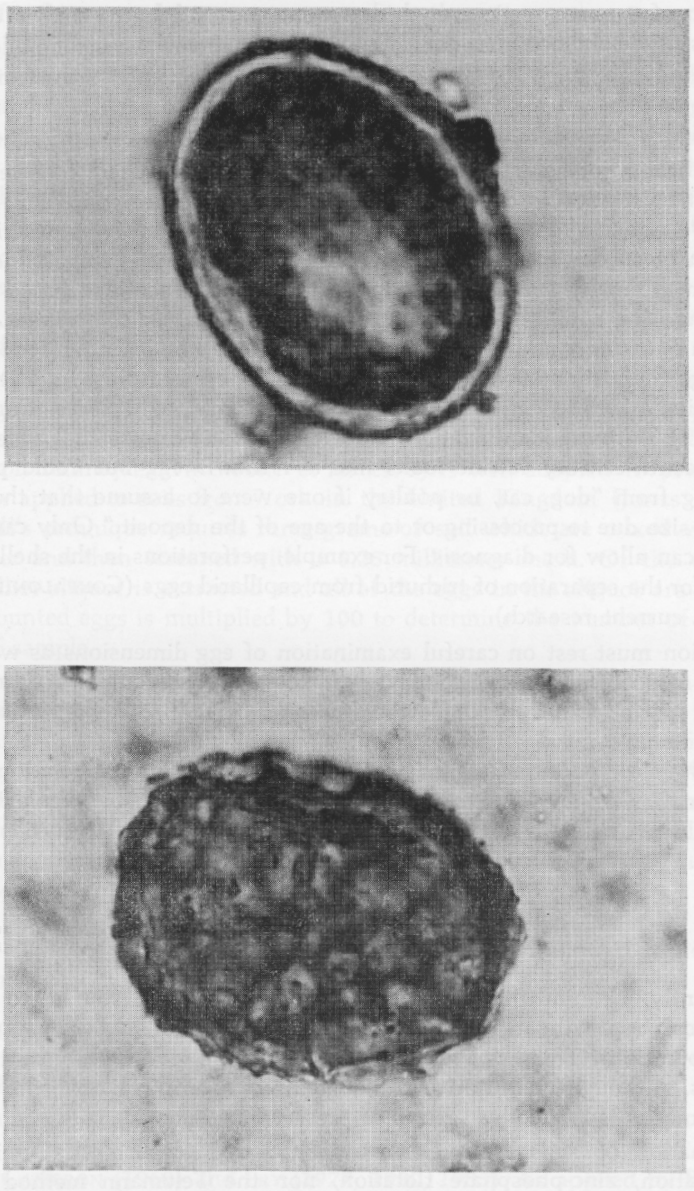


Fig. 9: Comparison of ASCARIS eggs recovered from the Queen Anne Square latrines by palynological processing (below) and sodium chloride flotation (above). Note the reconstituted surface coat of the egg recovered by palynological processing. No ASCARIS egg recovered by flotation exhibited similar surface sculpturing.

noted until the acetolysis treatment. This stage of the process destroyed the eggs of *A. lumbricoides* and *S. iapanicum*. Many *T. pisiformis* eggs were destroyed by the process. The ratio of *C. sinensis* rose after acetolysis which indicates that this species was not as badly effected by the treatment. A second sample of feces was treated with potassium hydroxide. No change in proportion or egg number was noted after this treatment.

This experimental treatment demonstrates that processing soils with pollen extraction techniques is not an ideal way to study helminth eggs from latrine soils. The fact the technique did work in the Elden Pueblo and Queen Anne Square analysis is probably due to the high cellulose and hemicellulose content of the soils which neutralized the acetolysis solution before complete destruction of helminth eggs occurred. The acetolysis solution of 9 parts acetic anhydride to one part sulphuric acid is designed primarily to dissolve cellulose and hemicellulose. A beneficial aspect of pollen extraction procedure is that the surviving eggs are cleaned and consequently surface morphological features are better seen (Figure 9). If this technique is used, another technique should also be applied to ensure that eggs have not been destroyed.

The identification of helminth eggs can be problematical. There is a certain amount of intraspecific variation in egg shape and size that can cause confusion. With certain taxa, eggs are not used as taxonomic tools and are not extensively described to allow identification of species based on eggs alone. For example, it is impossible to differentiate between the many species in the tapeworm family Taeniidae based on egg morphology alone. The similarity in structure between *A. lumbricoides* and *A. suum* and also between *T. trichiura* and *T. suis* hampered the ability of TAYLOR (1955) to associate helminth eggs found in a latrine with humans as opposed to pigs. In other cases, there exist close similarities in egg morphology between unrelated species. Commenting on the problem of *Trichuris* identification, GOOCH (1983: 206) notes that an egg recovered from a thirteenth-century latrine looks like a *Trichuris* egg but could possible be a capillarid egg from "dog, cat, or poultry if one were to assume that there had been distortion in size due to processing or to the age of the deposit." Only careful description of eggs can allow for diagnosis. For example, perforations in the shell of capillarid eggs allows for the separation of trichurid from capillarid eggs (CONFALONIERI, FERREIRA, and ARAUJO, current research).

Identification must rest on careful examination of egg dimensions as well as surface morphology. Also of importance is the archaeological association of latrine deposits. Artifacts or special architectural features such as barrel latrines (MOORE 1981) present strong circumstantial evidence that cess deposits are human associated. Finally, the find of human specific parasites in levels containing other helminth remains demonstrates human origin. For example, the find of *Enterobius vermicularis* eggs in the soils from Elden Pueblo is circumstancial evidence that other trichurid, ascarid, and cestode eggs in the same levels resulted from human parasitic infections (Figure 8).

Quantification of Helminth Remains from Latrine Soils

Very recently, helminth studies from latrine soils have sought to establish of epidemiological approach and to provide for comparative studies (HERRMANN 1986, 1987, in preparation). This has required a change in the techniques used to study eggs from latrines. Although quantitative clinical techniques are available, the effectiveness of some techniques is limited by aspects of preservation.

In examination of soils from Germany, HERRMANN reports that neither sodium chloride flotation, zinc-phosphate flotation, nor the Telemann method (also called MIF-ether concentration) are effective in concentrating eggs (MELVIN and BROOKE 1974: 30-31; THIENPONT et al. 1979: 35). Further experimentation was done with a discontinuous sucrose gradient solution (100/0, 30%, and 54%) spun

at 23,000 r. p. m. in a centrifuge (rotor 6 x 38) for 30 minutes. *Trichuris* eggs were found equally distributed throughout the centrifuge tube. *Ascaris* eggs were concentrated in the 30%-54% contact area and within the 54% sucrose. Thus is was concluded that concentration of helminth eggs is not usefully done by ultracentrafugation (i. e. by sequential flotation techniques).

The German soils were also treated with geological sedimentation techniques. The techniqques are designed to concentrate specimens of specific dimensions. The soils were sedimented for objects sized less than 20 micrometers. The technique was overly time consuming and not that effective. Acetolysis as used by palynologists was also attempted. This technique was found to destroy at least *Ascaris* eggs and is not recommended for future work.

Ultimately, an effective technique was innovated by H. H. Krüger for use with the German latrine soils. One or two grams of soil is mixed with 10 millileters of a solution consisting of four parts water and one part of concentrated detergent solution as used in commercial dishwashers. It may be necessary to dissaggregate the solution mechanically. The soil is soaked in the solution between one hour and two days, after which time the sample is centrifuged at 3,000 r. p. m. for one minute. The supernatant is washed with water, aggitated, and centrifuged four successive times. After the final water wash, water is added to the centrifuge tube, the tube is agitated and the contents are screened. The screened material is examined for helminth eggs and egg per gram counts are made. Soil samples that are still moist when excavated are preferred since dry soil samples show diminished egg counts.

Quantification of eggs in latrine soils has also been accoplished either by direct count of eggs in a gram of sediment (TAYLOR 1955; PIKE 1967) count of eggs in palynological extraction sediments (REINHARD et al. 1986) or by utilizing dilution techniques. The Stoll's Dilution technique (THIENPONT et al. 1979) is well adapted to prehistoric soils and has been applied to soils from York in the United Kingdom (JONES 1985). JONES' modified Stoll's technique requires three grams of soil which are mixed with 42 milliliters of water and then screened with a 0.25 millimeter mesh. Of the 42 milliliters, an 0.15 milliliter aliquot is extracted and all of the eggs in the aliquot are counted. The number of counted eggs is multiplied by 100 to determine the number of eggs present in a gram of sample.

With regard to quantification, egg counts per gram of soil depend on the depth from which the soil sample was taken as exhibited by latrine soils examined from german latrines (HERRMANN 1986). The distribution of eggs in a Medieval latrine from southern Germany is presented in Figure 10. This latrine dates to the 15th century AD. Although there are not many latrines of this size found undisturbed in central European towns, quite often smaller latrines or the lower parts of larger latrines are found. It is obvious from examination of Figure 10 that the number of eggs increases the further up the soil sample was taken.

Coprolite Analysis

Three stages of coprolite analysis relating to helminth study are rehydration, processing, and examination. The term coprolite is applied to desiccated as well as mineralized feces (BRYANT and WILLIAMS-DEAN 1975).

Rehydration in 0.5% trisodium phosphate is now a standard technique. VAN CLEAVE and Ross (1947) first developed the technique which was applied by CALLEN (1967L CALLEN and CAMERON (1960) and FRY (1977, 1980, 1985). As applied by REINHARD, the fecal sample is placed in the solution for 24 to 72 hours. After this time the reconstituted feces can be dis aggregated with a magnetic stirrer and screened using a fine water jet.

Experimentation with other rehydration techniques has been accomplished by SAMUELS (1965).

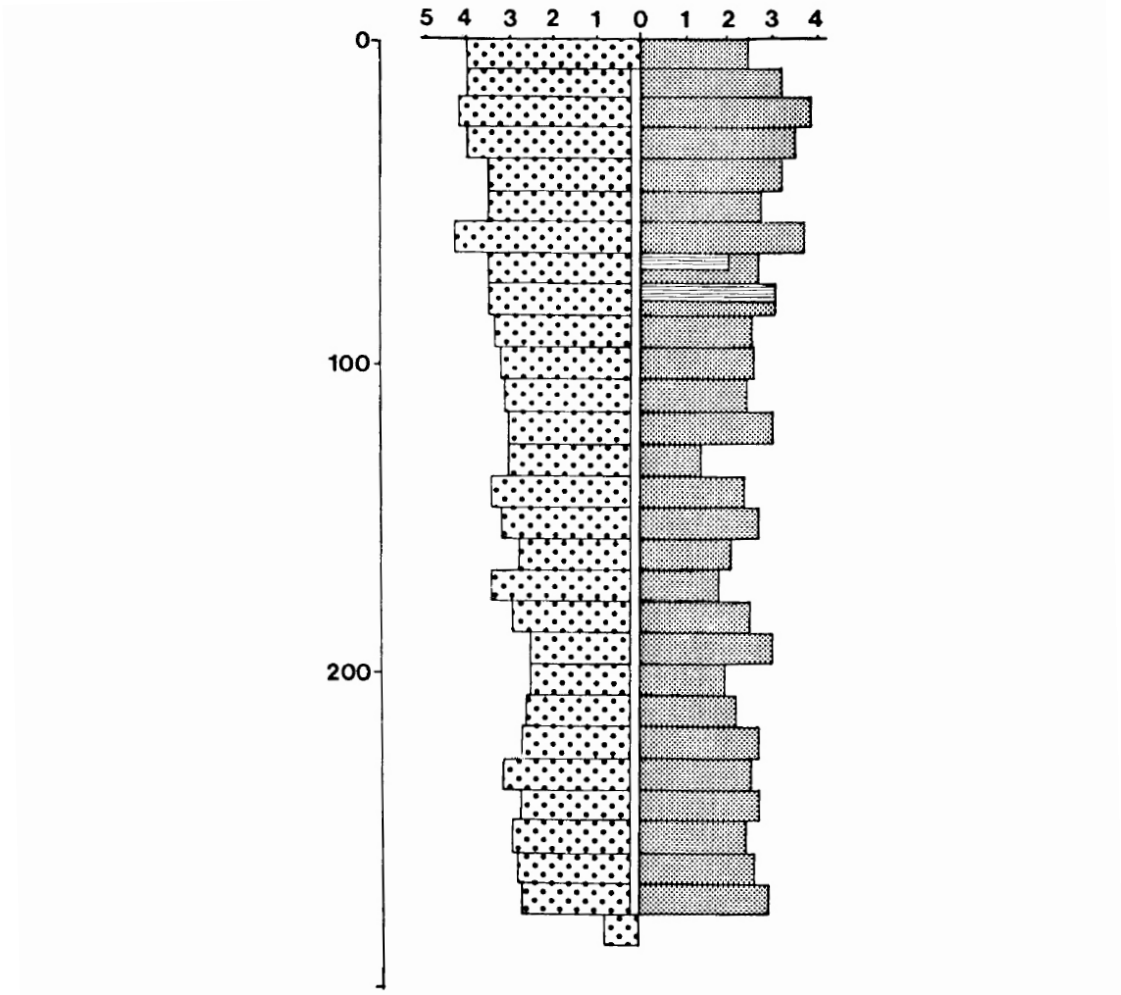


Fig. 10: Relation between egg counts and depth of a medieval latrine from Freiburg Abbey, West Germany dating ot the 15th century. Note that the egg count increases with dereasing depth. Top scale is egg count given in log naturalis, left scale is depth of the sample. Small stipples indicate ASCARIS count, large stipples indicate TRICHURIS count, horizontal lines indicate DIPHYLLOBOTHRIUM count, and clear areas indicate FASCIOLA count. Fasc'ola eggs are present in all levels but in very small numbers, where-as DIPHYLLOBOTHRIUM is found in only two adjacent strata.

SAMUELS reports that, in specific application to helminth eggs, 0.5% trisodium phosphate was most efficient and does not disfigure helminth eggs. This technique has also been applied successfully to colon contents from desiccated mummies (FOUANT 1981).

In examination of 324 coprolites from Salmon Ruin, Turkey Pen Cave, Dust Devil Cave, Chaco Canyon and Antelope House from the southwestern United States, REINHARD found the trisodium phosphate technique ideal for the rehydration of feces. In the case of Salmon Ruin, the feces were autodaved before rehydration. This sterilization process was done to prevent possible infection with fungal pathogens that were thought to be present in the coprolites. Initially, there seemed to be no detrimental effect of the autoclave on helminth remains. However, the embryos initially preserved in pinworm eggs rapidly decomposed once mounted on microscope slides for analysis.

Bacterial and fungal decomposition was noted in some feces from sites other than Salmon Ruin. Af-

ter 24 hours, the odor of decomposition became evident in some rehydrating feces. To counter decomposition, acetic formalin alcohol (A.F.A.) was added to the feces. The addition of this preservative had the effect of halting decomposition. Acetic formalin is used to retard fungal and bacterial growth by other researchers (ARAUJO et al. 1981) who note decomposition after 72 hours. In the case of the autoclaved Salmon Ruin feces, no decomposition was noted in any of the 112 feces studied, even after 120 hours of rehydration without preservative added. Usually, rehydration for 48 hours is sufficient to fully reconstitute coprolites.

Occasionally, mineralized coprolites have been recovered. JONES (1983) experimented with several disaggregation fluids. Water, sodium hydroxide, and dilute hydrochloric acid solutions were tried. Only hydrochloric acid successfully disaggregated the mineralised feces to permit effective parasitological analysis. Recently, several mineralized alligator coprolites dating to 7,000 years B. P. were submitted for parasitological analysis (REINHARD, current research). Rehydration in trisodium phosphate was attempted. The feces did not respond to this solution. A few drops of hydrochloric acid were added to each sample. Immediately upon the addition of the hydrochloric acid, the feces began to dis aggregated (REINHARD, current research). In JONES' study, it was determined that the hydrochloric acid had no effect on the size of helminth eggs.

Conservation of coprolites for future research should be a consideration for paleoparasitologists. Samples as small as 0.5 grams can be successfully rehydrated and analyzed for helminth remains. The utilization of such small portions of coprolites ensures that sufficient portions remain for future studies. However, it is probable that some infections are missed by using small coprolite fragments for analysis as found by CONFALONIERI.

Important research in the concentration of helminth eggs from coprolites was pioneered by HALL (1972) and applied by FRY (1977). Two techniques were devised, each derived from clinical methods. These techniques are zinc sulphate flotation and formalin-ether concentration. The formalin-ether technique was applied to archaic feces Dust Devil Cave (REINHARD et al. 1985). In this case, simple sedimentation proved more effective than the formalin-ether technique.

Utilizing feces from Antelope House (REINHARD 1985b), more extensive experimentation with zinc-sulphate was done. Fecal samples containing the eggs of pinworm, *Trichostrongylus*, hymenolepidid, and also *Strongyloides* larvae were found in examination of coprolite sediments. Samples of each of these were treated with the zincsulphate technique. All tests proved negative for helminth remains. This test indicates that zinc sulphate flotation is not effective when applied to some rehydrated coprolites.

To test whether preservation in A.F.A. hindered the buoyancy of the eggs. 50 eggs each of *Protocephalus arcticus*, pseudophyllidian cestodes, and *A. lumbricoides* were soaked for one month in A.F.A. and then added to rehydrated coprolite sediments. Of the cestode eggs, all were recovered. The roundworm eggs, however, adhered to beavier fragments of fecal debris or the sides of the centrifuge tube and were not recovered. Only increasing the specific gravity of the solution resulted in the recovery of some roundworm eggs. However, with the increased specific gravity, much of the botanical detritus, fungal spores, and other debris also floated. This situation negated the practicality of using the high specific gravity solution.

The experiment indicates that although zinc-sulphate is applicable to modern feces, and some coprolites, it should not be applied to all coprolites. Microscopic examiniation shows that in some coprolites helminth eggs are collapsed, cracked, and in the case of hymenolepidids, decorticated (Figure 11). Such structural damage probably inhibits the buoyancy of some eggs.

One detrimental aspect of the zinc-sulphate solution noted by HALL (1972), FRY (1977), and verified by REINHARD'S research, was that eggs collapsed under the solution's heavy osmotic pressure, rendering the eggs nearly unidentifiable. Experimentation with this technique shows it to be an unreliable method of recovering parasite eggs from prehistoric feces.

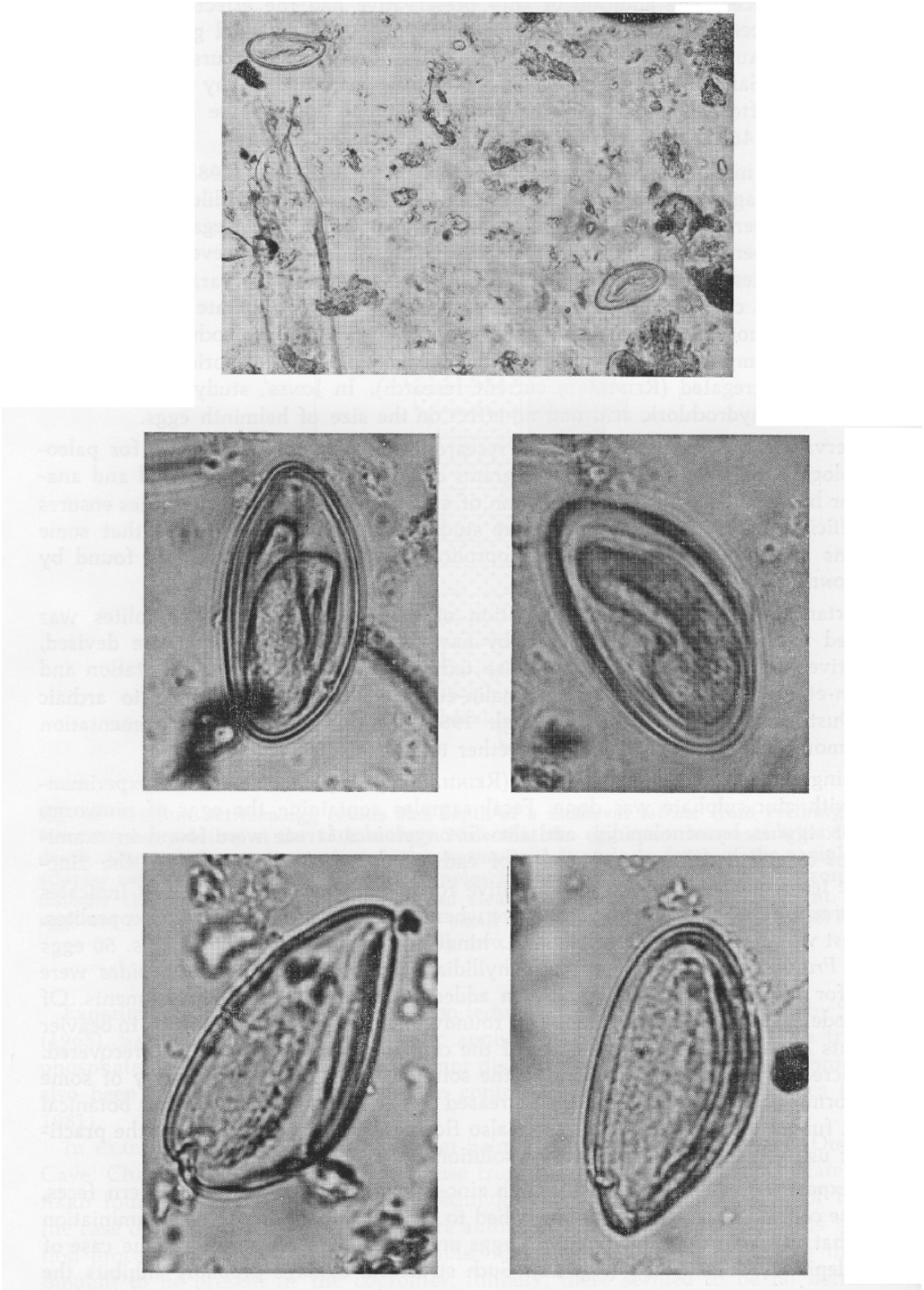


Fig. 11: Parasite eggs recovered from Antelope House coprolites. The upper eggs are of a hymenolepidid tapeworm. The outer capsule of the eggs has been lost resulting in the find of only the inner onchospere and wall. The lower eggs probably from a trichostrongyle worm. Note the crack in the lower left egg and the collapsed wall of the lower right egg. Such structural damage inhibits the buoyancy of the eggs in flotation media.

One reliable, but time consuming technique is the screening of rehydrated coprolites followed by gravitational sedimentation. The 0.5 gram of coprolite, once reconstituted, is screened through a 0.5 millimeter screen. The material that collects on top of the screen is examined and dried. The solution which passes through the screen is collected in a large beaker and then screened again through a 0.3 millimeter screen. The sediment resting on top of the screen is collected and preserved in A.F.A. in a small vial for examination. The solution passing through the screen, which contains smaller microscopic debris, is collected and centrifuged at 1,200 r.p.m. in a 50 millimeter centrifuge tube. After the supernatant is poured off, the smaller fecal debris transferred to a small vial in A.F.A. and allowed to settle. The upper layers of the sediment contain light plant and fungal remains as well as helminth eggs and larvae. Very delicate eggs can be recovered by this technique, even when partly decomposed (Figure 12).

An alternate method that is very successful in the recovery of both helminth eggs and larvae is the Lutz spontaneous sedimentation technique used by researchers in Brazil (ARAUJO et al. 1980; MASTRANDREA et al. 1967). After rehydration in trisodium phosphate, the feces are placed in a conical flask and allowed to sediment by gravity. The recent finds of helminth eggs and nematode larvae in excellent preservation testifies to the effectiveness of the technique (ARAUJO et al. 1980; FERREIRA et al. 1981, 1983a).

Mounting sediments for microscopic examination presents certain problems. HALL et al. (1983) note that many common mounting media, including silicone oil and glycerine, cause distortion of egg measurements. Water with formalin seems to be the best material for microscope preparations (CONFALONIERI). Although microscope slides can be sealed with commercial nail polish (REINHARD, HERRMANN), a more effective seal is a combination of petroleum resin mixed with bees wax or paraffin (CONFALONIERI, FERREIRA, and ARAUJO).

The same question posed by GOOCH (1983) concering the ability to identify parasites in latrine soils is relevant to the study of coprolites. Certainly some egg types such as the pinworm are so distinctive that confusion should not occur. However, some egg types do allow for confusion. For example, *Trichostrongylus* eggs can be confused with the eggs of ancylostomid hookworms in general shape. Only careful micrometer measurement allows for definitive diagnosis. With regard to the utility of using micrometer measurements in the identification of trichurids, CONFALONIERI et al. (1985) desiccated eggs of Trichiura and then rehydrated them in trisodium phosphate. This research indicates that what little shrinkage occurs is not sufficient to result in confusion of this human parasitic species with non-human parasites.

The identification of non-human parasites is problematical. Sometimes, careful research results in the identification of animal parasites. In the case of non-human coprolites recovered from Brazil, animals in the area were trapped. Feces were collected from the animals and helminths parasitizing the animals were examined. By comparing modern helminth eggs with the prehistoric eggs, a definitive identification was made (ARAUJO et al. 1980, 1982). Comparison of fecal morphology between ancient coprolites and modern animal feces determined the origin of the feces.

The greatest source of confusion in helminth studies from coprolites is in the identification of nematode larvae as parasitic or free-living. The problem is best exemplified by KLIKS' dispute of hookworm larvae identified from Brazil (KLIKS 1982, 1983; FERREIRA et al. 1980, 1983b). The eventual find of hookworm larvae in colon contents of a mummy confirmed the original identification (FERREIRA et al. 1983a).

The problem presented by nematode larvae identification has been addressed at length by REINHARD (1985a, 1985b) with respect to larvae recovered from Antelope House and by SAMUELS (1965) with respect to larvae from Mesa Verde coprolites. Both stress the importance of examining the coprolite for fungal growth, mite infestation, and beetle infestation which indicates how long the feces remained moist after defecation. Because dry feces present an inhospitable environment for free-living

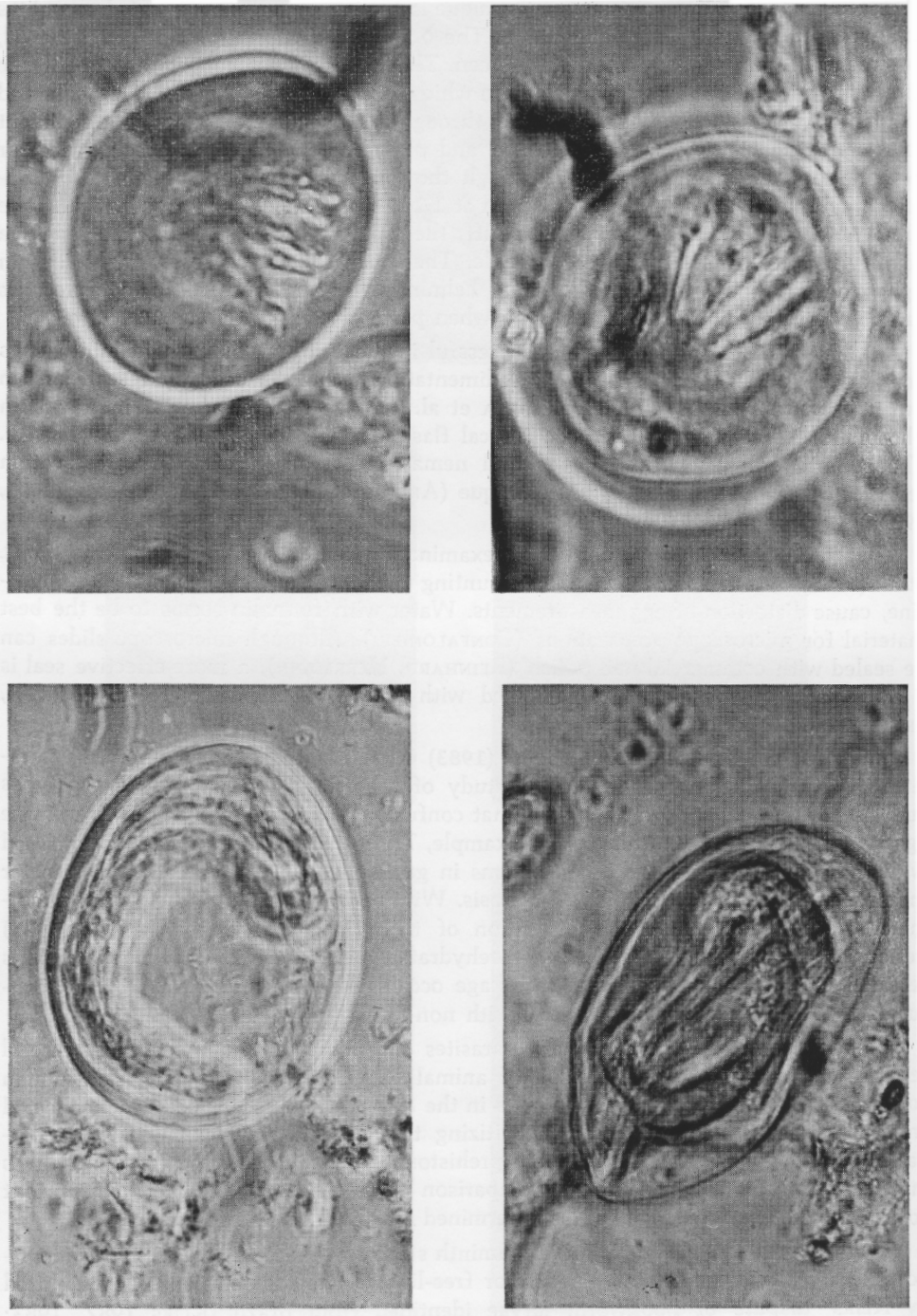


Fig. 12: Pinworm eggs recovered from coprolites by sedimentation. The upper three photographs are of eggs from Antelope House which were excellently preserved. The lower two photographs are of poorly preserved eggs from Pueblo Bonito, Chaco Canyon National Monument, New Mexico, USA. The fact that both well preserved and poorly preserved eggs are recovered by sedimentation testifies to the effectiveness of the technique.

nematodes, it is unlikely that feces which desiccated quickly would be infested. Also stressed is the importance of examining the coprolite prior to rehydration for bore holes or other evidence that animals from the cave environment entered the feces.

Examination of the worms can provide important clues regarding parasitic nature. When only first stage larvae are found, a parasite infection is implicated. With freeliving species it is likely that several larval stages as well as adults would be present. If the larval morphology is well preserved, morpholgical features may suggest whether the worms are parasitic or not.

Whether working with parasites derived from coprolites or latrine soils, the microscope is the main tool of the paleoparasitologist. The light microscope is suitable for most work. The transmission electron microscope has been used to examine the details of a taeniid tapeworm by HORNE (1983; MILLET et al. 1980: 81). HORNE, however, does not feel the transmission electron microscope will be of much use to paleopathologists since the ultra-structural detail of even well preserved material is lost.

The scanning electron microscope is of some value in certain cases. DALTON et al. (1976) utilized scanning electron microscopy in the examination of hookworm adults still attached to the intestine of a Peruvian nummy. HORNE (1983) utilized scanning electron microscopy in the examination of ectoparasites found in prehistoric Eskimo hair. In the REINHARD'S experience, scanning electron microscopy is of great value in determining the identity of dubious objects found in coprolites. For example, eggs of what appeared to be *Toxocara* sp. were found in some coprolites from Dust Devil Cave, Utah. Although three clinical parasitologists verified the *Toxocara* identification, and scanning elecron microscopy demonstrated that the objects were actually spores from a fungus in the family Endoganaceae. In the future, the scanning electron microscope may prove important in specific identification of helminth eggs and in examining the morphology of nematode larvae found in coprolites. Scanning electron microscopy is of useful in the morphological distinction of modern helminth eggs (KAZACOS and TUREK 1983).

When working with remains of prehistoric helminths from coprolites or latrine soils, it is important to stress HORNE'S (1985) recommendation that the researcher consult other members of the parasitological community in making identifications. This leads to more secure identifications. Consulting with both human, veterinary, and wildlife parasitologists is benefical. Often insight is available from one of these subfields that is not present in the others.

Summary

Standard techniques for the analysis of prehistoric soils have not been devised. It is unlikely that any single technique is applicable to all types of fecal remains. This is due to various environmental conditions which effect the preservation of helminth ova. In general, gravitational sedimentation is a useful technique for isolating helminth eggs and larvae from coprolites. Latrine soils pose greater problems for helminthological examination. Although various clinical techniques have been successfully utilized in soil study, it is important to remember that some latrine soils have not yielded helminth eggs to any clinical technique. Consequently the paleoparasitologist must be ready to innovate new techniques rather than depend on clinical techniques.

Beyond the problems of technique, what research done with identification of parasites is very encouraging. At this point it appears that the measurement and morphological characteristics used to identify modern parasites can also be applied to paleoparasites.

The trends of paleoparasitological research today emphasize experimentation and quantification as well as precise identification. In the future, these trends will lead to a more rigorous study of parasites in prehistory.

Zusammenfassung

Standardtechniken für prähistorisrhe Bodenanalysen existieren bisher nicht. Es ist unwahrscheinlich, daß irgendeine einzige Technik für alle Arten von Fäkalienreste angewandt werden kann. Das ist auf verschiedene Umweltbedingungen zurückzuführen, die den Erhaltungszustand von Parasiteneiern beeinflussen. Im allgemeinen ist Gravitationssedimentation eine nützliche Technik, urn Parasiteneier und Larven von Coproliten (Kotstein) zu isolieren. Latrinenböden stellen größere Probleme für helminthologische Untersuchungen dar. Obwohl verschiedene klinische Techniken erfolgreich für Bodenuntersuchungen angewandt wurden, ist es wichtig daran zu erinnern, daß einige Latrinenböden mit keiner klinischen Technik Parasiteneier erbracht haben. Infolgedessen muß der Paläoparasitologe bereit sein, eher neue Techniken einzuführen als an klinichen Techniken festzuhalten.

Abgesehen von den Problemen der Technik ist es sehr ermutigend zu sehen, welche Untersuchungen durch Identifikation von Parasiten durchgeführt wurden. Hier scheint es, daß Messungen und morphologische Merkmale zur Identifizierung von heutigen Parasiten auch auf Paläoparasiten angewandt werden können.

Die Trends in der heutigen paläoparasitoligischen Forschung betonen Experimentieren und Quantifizieren ebenso wie genaue Identifikation. Zukünftig werden diese Tendenzen sicherlich zu einer gründlicheren Untersuchung von Parasiten in der Prähistorie führen.

Résumé

Jusqu'à présent des techniques standards pour des analyses du sol préhistorique n'existent pas. II est peu probable que l'on puisse appliquer une seule technique sur tous les types de restes fécaux a cause de différentes conditions de l'environnement qui effectuent la préservation des oeufs d'helminthes. En général la sédimentation de gravitation est une technique utile pour isoler des oeufs d'helmintes et des larves de coprolithes. Les fonds de latrines posent des problèmes plus grandes pour des examinations helminthologiques. Bien qu'on ait utilisé avec succés des techniques cliniques pour des analyses du sol il est important de rappeler que quelques fonds de latrines n'ont pas fourni des oeufs d'helminthes à l'aide des techniques cliniques. Par conséquent la paléoparasitologie doit être disposé à innover de nouvelles techniques plutôt que dépendre de techniques cliniques.

A côte des problèmes de la technique il est trés encourageant d'observer quelles recherches avec identification de parasites ont été faites. A ce point il paraît qu'on peut appliquer des mesures et des caractères morphologiques, utilisés pour identifier des parasites modernes, aussi sur des paléoparasites. Les tendances de la recherche paléoparasitologique d'aujourd'hui soulignent l'experimentation et la quantification aussi bien que l'identification précise. Désormais, ces tendances méneront sans doutes à des recherches plus profondes de parasites dans la préhistoire.

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