

mined, sufficient amounts of 5 per cent acetic acid (Fig. 2, E), 0.1 N hydrochloric acid (D), 5 per cent monochloroacetic acid (C), and 5 per cent dichloroacetic acid (B), respectively. The ability of these acids to increase the sensitivity and range of this method *decreased* in the following order: trichloroacetic, dichloroacetic, monochloroacetic, hydrochloric, and acetic acid. This would suggest that the specificity of trichloroacetic acid in affecting this method is a function of the number of chlorine atoms attached to the carbon adjacent to the carboxyl group and not a matter of pH or the acetate radical itself.

References

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Use of Phenol Formaldehyde and Vinyl Resins in Sealing Liquid Mounting Media on Microscope Slides

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A persistent difficulty in the preparation and preservation of certain types of plant materials on microscope slides is the problem of permanently sealing liquid mounting media. Glycerin, lactic acid, lactophenol, and certain other mounting fluids of diverse composition are exceedingly difficult to inure against changes in atmospheric humidity, temperature, and slow chemical reaction with the sealing agent. The dependence upon liquid media for mounting certain plant tissues, although unfortunate, is necessitated by requisite indices of refraction and other physical and chemical properties of diverse mounting fluids. It therefore becomes necessary to employ a sealing compound which gives a reasonable promise of permanence if extensive collections of such plant materials as pollen grains, leaf cuticles, fungus mycelia, and other whole mounts are to be maintained for permanent reference.

Recently, in the preparation of reference collections of root and leaf epidermal tissues for use in the identification of plant fragments in peat and lignitic coals, a need was realized for permanent sealing of glycerin and lactic acid preparations of such tissues. Consideration of the physical and chemical behavior of various synthetic resins and plastics indicated the possibility that phenol formaldehyde and vinyl resins might provide the necessary physical properties and chemical resistance. Preliminary tests showed a p-phenyl phenol formaldehyde resin and a vinyl acetate resin to be very promising for prolonged sealing of noncorrosive and certain corrosive media. These resins, when applied copiously with a brush or pipette to clean glass surfaces, form an effective seal which is not altered by prolonged storage at normal temperatures. In the case of the phenolic resin an accelerated aging test involving 8 months storage at 50° C. with frequent intermittent changes to room temperature has resulted in perfect sealing of lactic acid and glycerin preparations. The vinyl resins have not been subjected to as rigorous tests, but appear to be equally effective.

The phenolic resin showing the most desirable properties for a sealing agent is a p-phenyl phenol formaldehyde compound containing tung oil and a metallic soap as oxidizing agent. The

resin is prepared and sold as a commercial varnish and has the syrupy consistency of a thick oil. The setting of the liquid to a solid is complex chemically, involving solvent evaporation, oxidation of the tung oil, formation of colloidal systems, and polymerization of the resin. The dried and polymerized resin is exceedingly hard, yet slightly elastic, shows extreme adhesiveness to glass, and is extraordinarily inert chemically. When cured by drying and especially by heating to about 50° C., the resin is completely inert to ordinary organic solvents such as alcohols, hydrocarbon solvents, and halogenated hydrocarbons. Resistance to mineral acids was tested by ringing lignin residues of wood sections prepared with 72 per cent sulfuric acid. The resin shows effective sealing of the strong acid after 3 months storage at 40° C. A slight carbonization at the edges of the cover glass occurred shortly after sealing, presumably by action on the tung oil. In the case of organic acid media such as lactic acid and lactophenol, clouding of the resin may occur near the interface with the mounting fluid. This cloudiness does not appear to progress after polymerization of the resin at 50° C., and no tendency has been observed for the mounting fluid to become cloudy by emulsification of the sealing agent, as is frequently the case with compounds such as Noyers cement after prolonged storage.

For best results the p-phenyl phenol formaldehyde resins should be kept in a large stock container of quart capacity or more and drawn off only when needed. Small portions of the liquid resin, when kept in dropping bottles or similar containers, set to a gel within a day or two and are useless after jelling since the material becomes insoluble in organic solvents. When still liquid, the resin may be thinned with neutral xylene or ethyl acetate. The tendency to gel after exposure to air can be obviated by proper handling.

Slides, after ringing with the resin, may be dried for 8-24 hours and placed on a warming table or in an incubator to complete the polymerization of the resin. Some change in the color of the resin accompanies polymerization, resulting in an amber yellow. The resin shows no wrinkling by shrinkage after slow drying and polymerization on the glass. Clean glass surfaces are essential for perfect sealing, although excess glycerin or other liquid at the edges of the cover glasses may be effectively sealed if it is not sufficiently copious to form a film between resin and glass.

Phenolic resins of the type described here are produced by the Bakelite Corporation, New York City. The commercial product used in the writer's laboratory is prepared by the Brooklyn Varnish Company under the trade name of Tufon #74.

The vinyl resins tested as sealing agents showed great variation in physical properties and resistance to mounting media. Their advantages are ease of solubility in a wide range of organic solvents and clear, glass-like appearance. A vinyl acetate resin (Bakelite Corporation No. AYAF) proved most useful as a sealing compound, although its texture, chemical inertness, and adhesive properties are inferior to phenolic resins. It may be dissolved to desired viscosity in 95 per cent ethyl alcohol, butyl acetate, dioxane, ethylene dichloride, and other organic solvents. The vinyl acetate polymer appears to be inert to glycerin. The solubility of certain vinyl resins in ethyl alcohol containing as much as 50 per cent water suggests its use in the preparation and embedding of whole mounts of plant and animal tissues in which complete dehydration should

be avoided. Vinyl acetate resins are thermoplastic and remain somewhat flexible at room temperature.

Brief experience with the physical and chemical properties of certain industrial plastics has suggested that many other uses for such compounds may be found in histological techniques adapted for plant tissues. Reliance on the tried and true methods need not be sacrificed in the development of these new techniques or new materials for older techniques.

An Effective and Nontraumatic Method of Handling Monkeys

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A technique which we use for catching monkeys preparatory to administering anesthesia has attracted sufficient favorable comment from monkey handlers who have witnessed its operation to warrant a brief note.

The two essential items of equipment are (1) the transport cage and (2) the square-hoop net, illustrated in Fig. 1. The

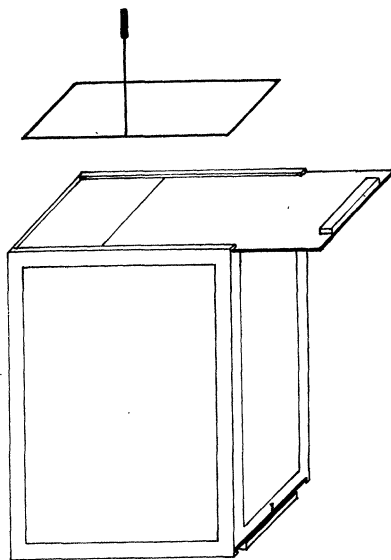


FIG. 1

transport cage, which measures 13 x 16 x 20 inches, is equipped with a sliding door on each end; its sides may be made of wire mesh to facilitate observation. The hoop of the net is constructed of $\frac{3}{8}$ -inch rod. Its size (10 x 13 inches) is such that it fits easily into the transport cage.

The first step in the procedure is to chase or lure the monkey into the cage. This can be accomplished with surprising ease, for rhesus monkeys, after a little experience with the cage, react to it almost as though it were a haven of refuge. Once the animal is inside, the cage is set on end. The net is then placed in position over the sliding door, which is now on top. When the door is withdrawn, the net is moved down over the monkey. The lower door is then withdrawn and the hoop held against the floor. When the cage itself is lifted off the net, the enmeshed monkey lies helpless at one's feet.

The entire procedure is tolerated with a minimum of emotional upset for both monkey and man. Using this technique, a single unassisted worker can carry out intraperitoneal or subcutaneous injections. The various uses to which the method may be put are self-evident.

Preservation of *Plasmodium vivax* by Freezing

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There are several methods of fever therapy for neurosyphilis, but induced malaria, either alone or combined with other agents, remains the method of choice of many syphilotherapists. A serious limitation to the more general use of malaria therapy has been the difficulty of providing a constant, readily available source of parasites. Plasmodia do not remain viable in blood under the usual conditions of storage (room temperature and icebox) for more than a few days (4). Preliminary results of preserving *Plasmodium vivax* by low-temperature freezing suggest that this may prove a practical method of long-term preservation.

Preservation of protozoa by freezing is not new. Turner (5) was able to maintain *Treponema pallidum* and *pertenue* in a viable state at low temperatures for long periods of time. Coggeshall (1), studying human, avian, and monkey malaria parasites, reported failure with a process involving rapid freezing at -72° to -80° C. and slow thawing, but when thawing was effected rapidly, monkey parasites were preserved successfully for as long as 70 days. Others (2, 3, 7) have been able to preserve the various parasites of bird malaria at temperatures of -50° to -70° C. for long periods of time. Recently Weinman and McAllister (6) have reported the freezing and prolonged storage of several types of pathogenic human protozoa with conservation of virulence. Although human plasmodia were not studied, the preservation of *P. lophurae* in the frozen state for long periods was confirmed. Russell (4) has alluded vaguely to low-temperature freezing of human malaria parasites as a means of preservation for use in therapeutic malaria, but provided no documentation.

Since we have been unable to find reports of preservation of human plasmodia by freezing techniques, we wish to record the successful transmission of malaria to three subjects by inoculation of blood infected with *P. vivax* which had been frozen and maintained at low temperatures for periods of 10-15 days.

The donor from whom the strain of parasites was originally obtained was H. K., a veteran of service in New Guinea, who had been suffering from recurring attacks of vivax malaria. At the time of bleeding, blood smears showed many young ring forms of *P. vivax*; a rough estimate of parasite density was approximately 10,000/mm.³. Twenty cc. of blood was withdrawn into 3 cc. of 4 per cent sodium citrate solution, of which 10 cc. was immediately injected intravenously into D. M., a neurosyphilitic receiving penicillin therapy. Five cc. of the remainder was transferred to a thin-walled glass test tube and rapidly frozen at a temperature of -75° C. by immersion in alcohol-dry ice mixture, the tube being rotated by hand until