

seals the whole to the jar. Extensive work by the termites in the mass often breaks it loose from the walls of the container unless it is held by the cap.

Altered sawdust is indicated theoretically by the findings of Roessler (4) and Hendee (1) that termites are dependent upon fungus for certain food elements. Cultures using sawdust from sound wood have proven uniformly unsuccessful. The choice of Monterey pine was one of convenience, since most of the termites cultured in earlier experiments were from logs of this species.

Many modifications of this procedure have been tried using thinner or heavier agar, different sawdusts, and combinations of soil and sawdust in layers, and using various fungicides in an attempt to prevent excessive growth of microorganisms. One modification involving the replacement of the agar cap with a layer of paraffin was unsuccessful in general because it caused early drying out. There seems some reason to believe that it may prove useful in culturing dry-wood termites. Further information regarding the standard method, its modifications, and the results obtained by it will appear elsewhere.

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An Improved Funnel Design

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A useful improvement on funnels for transferring liquids is obtained by cutting off most of the stem of a pyrex funnel and sealing on a standard-taper, ground-glass joint. Since no reference to such design has been seen in the literature, it is believed worthy of description.

The advantages of using such a funnel are several: A stable funnel-bottle combination is achieved in which the funnel does not tip or move when used for filling flasks and bottles. No funnel stem is immersed in the liquid. If the bottle has been overfilled, the excess liquid, instead of overflowing from the bottle as with the usual funnel, merely fills the funnel. Liquid is not wasted and, when permissible, can be poured back into the original container. The funnel does not have to be raised to allow air to escape from the bottle, the diameter of the joint being large enough to let the air pass up through the center while the liquid passes down the wall. The result is a funnel that permits much faster flow than any yet used.

The 65-mm. and 100-mm. pyrex funnels sealed to $\text{F } 24/40$ ground joints have proved most satisfactory for average use. The ground joint is cut off about $\frac{1}{2}$ inch above the ground portion, and the funnel stem is removed; the funnel is cut high enough to allow easy mating with the joint. The two pieces are heated in a large, gas-oxygen flame until they are both quite soft; they are then joined, using slight pressure. Although no attempt is made to "blow" the joint, the seal should be complete and without pinholes. Careful annealing will remove strains; and the resulting joint is strong enough for most purposes.

Use of Radioactive Iodine as a Tracer in the Study of the Physiology of Teeth

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Although *in vitro* and *in vivo* dye studies (2) have proved valuable in research in dental physiology, such studies have limited applicability. Neither the surgical introduction of complex molecular dyes to teeth *in vivo* (3) nor the use of extracted teeth with accompanying permeability changes (4) provides an adequate demonstration of normal distribution of tissue fluid solutes in teeth.

The use of radioautography with radioactive iodine is presented as a method which clearly indicates the distribution of tissue fluid solutes in teeth under normal environmental conditions. Radioactive isotopes have been used in dental research since 1935 (5), but the results to date have been obtained with substances metabolized by teeth. While the need for studying metabolic processes is evident, a basic understanding of the pattern of distribution of tissue fluid solutes through the tooth tissues is fundamental.

Iodine was chosen as a suitable tool to trace the distribution of tissue fluid solutes in teeth because (1) it is highly soluble in tissue fluid (1); (2) it has not been demonstrated with microchemical or spectroscopic analysis to be normally present in the enamel, dentine, or cementum; and (3) it has a readily available and easily utilized radioactive isotope.

To obtain a histologic correlation of the location of radioactive iodine with the microscopic anatomy of the tooth, radioautography was utilized. At best, other methods indicate concentration of the material in a general area (10), whereas with radioautography specific distribution may be studied.

¹³¹I in the form of NaI in physiologic saline solution was injected intraperitoneally into four cats and two rats. Approximately 0.5 millicurie/kg. of body weight was used. After 12 hours a blood sample was drawn, the animal put to death with ether, and the teeth removed intact. The blood sample was taken to establish the concentration of radioactive iodine in the blood at the time of sacrifice. Median longitudinal parallel sections of the teeth were made by grinding under oil on abrasive wheels to the thickness of 100-150 μ . The sections, after being buffed clean, dried, and weighed, were temporarily mounted on glass slides with Scotch tape in order to present one completely uncovered surface, and the activity determined by means of a Geiger counter. Assuming a uniform distribution of radioactive iodine and a negligible self-absorption of radiation, an estimate of the necessary exposure time was obtained, based on activity per unit area of section. Although this is not a quantitative method, it yielded highly satisfactory results.

In a darkroom the sections were placed directly in contact with the emulsion of Ansco No-Screen X-ray film and stored in cassettes and at the end of the calculated exposure time were removed from the film, which was developed under controlled conditions. The sections were then permanently mounted.

During the development of the technique Kodak medium lantern-slide plates, Ansco Triple-S Panchromatic film, and Kodachrome film for artificial light were also tried. A separate

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Geiger counter calibration must be made for each type of film used. The decision to use the No-Screen X-ray film was based on its high speed and relatively good contrast, with the realization that the large grain size might be a limiting factor in later work. When grain size is an important consideration, the medium lantern-slide plates are superior, but they require about triple the exposure time.

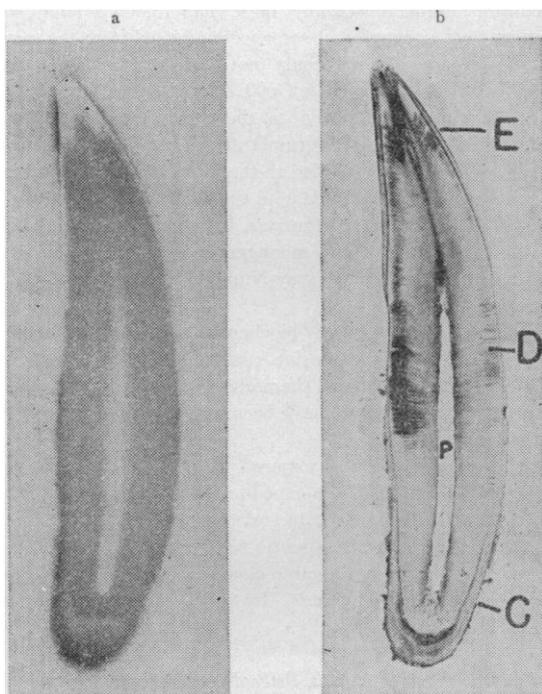


FIG. 1. (a) Radioautograph of a ground section of a canine tooth of an adult cat. (Artifact: scratch on emulsion at incisal edge.) (b) Photomicrograph of the ground section from which the radioautograph was made. (C, cementum; D, dentine; E, enamel; P, pulp.)

Fig. 1a is a radioautograph of a cat canine, the outlines of which can be clearly discerned over the background. Irregularities in the background are due to damages in the emulsion caused by the Scotch tape mounting. Fig. 1b is a photomicrograph (same magnification) of the ground section from which the radioautograph was made. Note that radioactive iodine is



FIG. 2. Radioautographs of ground sections of teeth of another adult cat.

present in the enamel, dentine, and cementum. In all figures the pulpal area is clear because the pulp was lost in grinding. In the dentine there is an even density except for an irregular distribution of radioactive iodine in the coronal and apical regions with bare areas proximal to the enamel and cementum. Radioactive iodine is seen to be distributed throughout the cementum with relatively high density in the outer portions and decreasing density near the dentine.

Fig. 2 illustrates radioautographs made from ground sections of teeth of another adult cat. These are presented to emphasize the variation in distribution of radioactive iodine under uncontrolled conditions.

Correlation between radioautographs and histologic structure depends upon detailed study of the ground sections as well as good radioautographs. This detailed study may be enhanced by utilizing staining techniques to bring out the histologic structure in the ground section.

To get the maximum information from the study of a radioautograph it is necessary to compare identically corresponding points in the autograph and the original section. This condition can best be approximated with the present technique by manually superimposing a negative of a photomicrograph of the section on one of the radioautograph. Evans (7) has recently developed a method of obtaining an automatically superimposed section and radioautograph for soft tissues. An adaption of this method for calcified tissue sections may be possible and would be highly desirable.

In view of the high solubility of iodides in tissue fluids and the evidence that some form of tissue fluid does exist in teeth (4), the radioactive iodine will appear in those portions of the tooth to which fluid is transported from the blood stream, by whatever mechanism. The radioautographs above show radioactive iodine uptake by the teeth, giving further indication that a dynamic fluid medium exists in the calcified structures.

The distinction between a metabolized substance and one such as iodine may be indicated by the comparison of our radioautographs with phosphorus radioautographs such as those published by Erbacher and Wannemacher and Berggren (6). The best ones seen by the authors were done by Bevelander and Amler (3), but these have not been published. The radioautographs show that the radioactive phosphorus concentration is highest in young teeth and is higher in those parts of adult teeth that have not matured. It can be readily seen from the radioactive iodine autographs that this distribution is not true of iodine.

At present the size of the dose of radioactive iodine necessary to obtain a satisfactory radioautograph has limited dental studies in humans to individuals receiving large doses for treatment of thyroid disorders (9). An attempt is being made to refine the technique sufficiently to obtain autographs from the administration of safe tracer doses to normal individuals.

The results obtained demonstrate the efficacy of the use of radioautography with radioactive iodine as a method for studying the physiology of the fluid in teeth in communication with the systemic circulation.

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