

nique. A histological diagnosis of a malignant neoplasm was made in each instance among the tabulated cases. The observation of fluorescence in the tumor was correlated with the ultimate diagnosis established by microscopic examination of the tumor (see Table 1). The majority of the 9 failures have occurred in attempts to fluoresce large, bulky, abdominal tumors. Carcinomata of the colon, stomach, and breast were found to be less likely to fluoresce. This might be related to the factors of dosage or the lack of a sufficient time interval between injection of the dye and examination. It has also been found that areas of edema and cyst formation will retain the dye for many hours. This is a source of confusion in attempts to fluoresce abdominal masses. In addition, it should be noted that necrotic tissue will not fluoresce, since dead cells are not stained by the dye.

The most consistent results have been obtained in the examination of brain tumors. Twelve cases suspected of having an intracranial neoplasm were examined with the fluorescein technique (Table 1). In subcortical lesions this test has been of

particular value. Tumor tissue secured from suspected areas by aspiration needle biopsies was readily recognized by the exaggerated fluorescence observed under ultraviolet light. Both the glioma and meningioma groups of brain tumors have been recognized correctly as tumor tissue by this method. In each case, the fluorescent areas have been subsequently proven neoplastic by standard methods of pathological examination.

An attempt has been made to quantitate by fluorometric methods the amount of dye present in both tumor and normal tissues after varying time intervals.

In additional studies, employing iodine substitution products of fluorescein, an increased visualization of certain mouse tumors by X-ray was noted. The possibility of using radioactive iodine in these substituted dyes has been considered.

References

1. DANCWORTT, P. W. *Luminiszenz-Analyse im filtrierten ultravioletten Licht*. Leipzig: Akademische Verlagsgesellschaft, 1934.
2. HERLY, L. *Cancer Res.*, 1944, 4, 227-231.

I N T H E L A B O R A T O R Y

Methods for Culturing Termites

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Progress in the study of the determination of castes in termites is dependent upon the development of satisfactory methods of culturing groups taken from both large colonies and incipient primary colonies (2).

Maintenance of groups of termites in the laboratory under conditions sufficiently standardized to permit a comparison of results necessitates: (1) a satisfactory source of food material; (2) the continued presence of moisture but avoidance of excessive moisture, especially free moisture; (3) visibility for observation without taking down the cultures; and (4) avoidance of conditions conducive to extensive development of microorganisms.

A method employing agar has been in use by the senior author for several years (3). The junior author has had much to do with the recent use and development of this method.

The standard procedure is as follows: A quantity of altered Monterey pine sawdust is first screened to remove very coarse wood chunks, bark, etc. and then sifted to remove fine, dust-like particles. To 2.8 grams of this sawdust, which is placed in the 1-ounce jar commonly used, is added 9 cc. of 3 per cent agar at a temperature low enough to prevent extensive absorption of agar by the particles of wood, yet high enough to allow complete mixing with the sawdust. Quantities are altered correspondingly for larger or smaller containers. When the agar is thoroughly mixed with the sawdust, the whole mass is pressed down firmly and leveled with the fingers. When the

sawdust-agar mass has cooled, with the lid off, 4 or 5 cc. of 4.5 per cent agar is added just hot enough to pour (it is desirable to avoid large bubbles in the cap). Following solidification of the agar cap a hole is punched to the bottom along the glass through cap and agar-sawdust mass. A cork border is convenient for this purpose, the size depending upon the size of the species to be used and of the group. For small species a hole punched with a wire rod is sufficient. It is important to make this hole along the glass in order to facilitate observations.

The termites, which are placed on the agar surface, will usually enter the hole at once and begin working in the sawdust-agar mass, also usually sealing up the hole and thus conserving the moisture.

To prevent the excessive development of microorganisms, the jars should be thoroughly cleaned and autoclaved or boiled, and the termites should be carefully selected and handled as gently as possible. All inactive or injured ones should be removed and exposure to the air reduced to a minimum. If some die on the agar surface, they should also be removed. For methods of handling termites, see Light and Illg (3).

The size of the container and the amount of culture medium must be regulated to suit the number of termites to be supported. Small groups of termites cannot effectively control excessive development of microorganisms if a large quantity of culture medium is isolated from the termites. Free moisture occurs most commonly if the jar is closed before the agar has cooled.

In the sawdust-agar mass the termites work largely against the glass, making it possible to observe changes in individuals or the group. The agar in the mass and the cap serves to hold water; the cap, prevents escape of moisture from below and

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.

References

1. HENDEE, ESTHER C. *Univ. Calif. Publ. Zool.*, 1933, 39, 111.
2. LIGHT, S. F. *Quart. Rev. Biol.*, 1942; 17, 312.
3. LIGHT, S. F., and ILLG, PAUL L. *Univ. Calif. Publ. Zool.*, 1945, 53, 1.
4. ROESSLER, ELIZABETH S. *Univ. Calif. Publ. Zool.*, 1932, 36, 357.

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 $\frac{1}{8}$ 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 plano-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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