is our impression that the assay for tissue glucuronidase will prove of more value in lower genital tract carcinoma.

In the presence of untreated lower genital tract carcinoma, the vaginal secretion was uniformly high in glucuronidase activity, the lower range being 477 γ uncentrifuged (Table 3). It is apparent from studies on vaginal secretion obtained from women with benign lesions that false positive tests occurred (Table 4). These were obtained principally from patients who were pregnant and from patients with a trichomonas vaginitis. Following irradiation therapy for genital carcinoma, the vaginal fluid was less active (in the absence of a recurrence) than in the untreated group. This observation suggests the use of vaginal fluid assays as a method of follow-up. Results from the centrifuged supernatant fluid were generally lower than those of the uncentrifuged specimen. Thus, it may be inferred that more glucuronidase activity was associated with the solid (cellular) component of the suspension. It was found that the centrifuged supernatant fluid of suspensions in Tyrode's solution was less active than suspensions in distilled water. This is probably due to less laking of the cellular component in Tyrode's solution. These studies are being continued on a larger scale.

TABLE 3

GLUCURONIDASE ACTIVITY IN VAGINAL FLUID OF UNTREATED CARCINOMA

| Identification | Microscopic diagnosis† | γ of β-Glucuronidase‡ | |
|---|---|---|---|
| | | Uncentrifuged | Centrifuged |
| 432565 | Sq. ca. cx. | 1458 | |
| | | 1368 | |
| 436773 | Sq. ca. cx. | 498 | 240 |
| 438049 | Sq. ca. cx. | 477 | 136 |
| 439485 | Sq. ca. cx. | 900 | 459 |
| 436773 | Sq. ca. cx. | 1335 | 1010 |
| 299510 | Sq. ca. cx. | 1412 | 962 |
| 140393 | Endomet. Car. | 1218 | 493 |
| A.M.* | Sq. ca. vag. | 883 | |
| 45424 | Sq. ca. cx. | 1930 | 665 |

* Patient from another hospital.

† Sq. ca. cx.—squamous carcinoma of cervix; endomet. car.—endometrial adenocarcinoma; sq. ca. vag.—squamous carcinoma of vagina.

‡ β-Glucuronidase expressed as γ of phenolphthalein liberated per ml of vaginal fluid per hr.

TABLE 4

GLUCURONIDASE IN VAGINAL FLUID OF BENIGN LESIONS

| γ of β-Glucuronidase* | Number examined | |
|---|---|---|
| | Uncentrifuged | Centrifuged |
| over 501 | 4 | 1 |
| 301–500 | 3 | 2 |
| 101–300 | 8 | 5 |
| 51–100 | 11 | 2 |
| 1– 50 | 17 | 19 |
| 0 | 7 | 23 |
| Total | 50 | 42 |

* β-Glucuronidase expressed in a frequency table as γ of phenolphthalein liberated per ml of vaginal fluid per hr.

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## The Nature of the Pigmented Sheath in *Drosophila* Tumors[1]


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In previous studies of tumors occurring in *Drosophila melanogaster* it has been noted that those in larvae several days old are surrounded by a pigmented coat, and appear as black, or brownish-black masses. In younger specimens, tumors, though present, lack the pigmented coat. It has been suggested by some workers (*2–4*) that the pigmented sheath is melanin, but this has not been substantiated by chemical tests.

The nature of the pigment is a matter of interest for several reasons. First, it seems to act as a limiting barrier against the growth of the tumor mass. Second, for detailed cytological study of tumors, it would be of great value if the pigment could be inhibited or delayed. Good cytological pictures may be obtained now only from tumors in quite young larvae.

Since melanin is the likeliest possibility in insect material, it was decided to test for it first, using two tests suggested by Cowdry (*1*). One is an oxidizing process employing $KMnO_4$ followed by oxalic acid; the other involves bleaching in the presence of concentrated NaOH.

Flies of tumorous strain "bw tu" were placed in half-pint containers, the caps of which held on their inner surfaces blocks of molasses agar seeded with yeast. The bottles were inverted and the flies could thus feed and deposit their eggs on the readily removable agar blocks. Caps were changed daily, the blocks each time being transferred to Petri dishes containing molasses agar well seeded with yeast. Here the eggs were allowed to hatch, and larvae to develop. Approximately 80–90 hr following transfer to the Petri dishes, tumerous larvae showed heavy deposits of pigment surrounding the neoplasms, and were considered ready for examination.

By flooding the Petri dishes with water, the larvae could be pipetted out and transferred to Syracuse watch glasses for microscopic study. Tumors were teased out with dissecting needles under broad field microscope and

[1] The work reported here is part of a research project supported by a grant from the Cancer Research Grants Branch, Division of Research Grants and Fellowships, National Institutes of Health, Bethesda, Maryland.

transferred by means of a fine pipette to Stender dishes containing either concentrated NaOH or 0.1% $KMnO_4$. At the end of 24 hr in NaOH, all tumors were found to be completely decolorized. This may be regarded as a positive reaction, since melanin is bleached by concentrated NaOH.

Tumors in $KMnO_4$ were allowed to remain for 24 hr, then rinsed in several changes of distilled water, and immersed in 0.3% oxalic acid. In all cases, after 12–24 hr in oxalic acid no trace of the dark masses could be found, the pigmented sheath evidently having been completely oxidized. This too may be regarded as a positive test.

Since the results in both cases are those expected of melanin, it would appear that the assumptions previously made concerning the nature of the pigment are correct.

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## An Internal Geiger Counter for the Assay of Low Specific Activity Samples of Carbon 14 and other Weak Beta Emitters in Biological Samples[1]


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The limiting factor in biological experiments with radioactive tracers is the great dilution of the isotope. Further increases in the specific activity of the isotopic compound are often impossible because of its limited availability or because of the biological effects of the radiations. Improvements in counting techniques offer the only practical solution. The procedure described here permits statistically valid analyses of samples having one-fifth or less the activity necessary for mica-window tubes.

The windowless Geiger tube of the continuous gas flow type shown in the accompanying figures (Figs. 1 and 2) permits the counting of all particles escaping from the sample. A satisfactory counting gas is a mixture of helium and alcohol vapor. The helium is bubbled through absolute ethyl alcohol kept at 0° C in an ice bath and the gas mixture passed through the counter.

The counter can be successfully operated with commercially available scaling circuits. The material to be assayed must be spread in fairly uniform thickness on a flat surface or shallow cup within a measured area. Samples of tissues are prepared by spreading an aqueous homogenate of the tissue on a flat copper disk of 2-in. diameter which has been cleaned with ether and oxidized in an oven at 160° C overnight. The spreading may conveniently be done while the copper disk is revolving at the rate of 5 to 20 rpm on a turntable and under a gentle current of dry air. Samples of blood, urine, and solutions or suspensions of water-soluble or insoluble

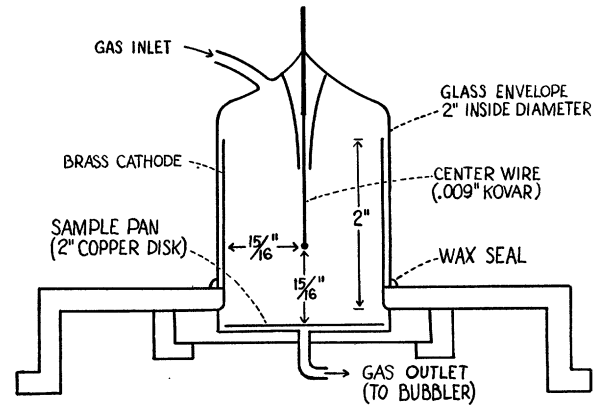


FIG. 1. Cross-sectional view.

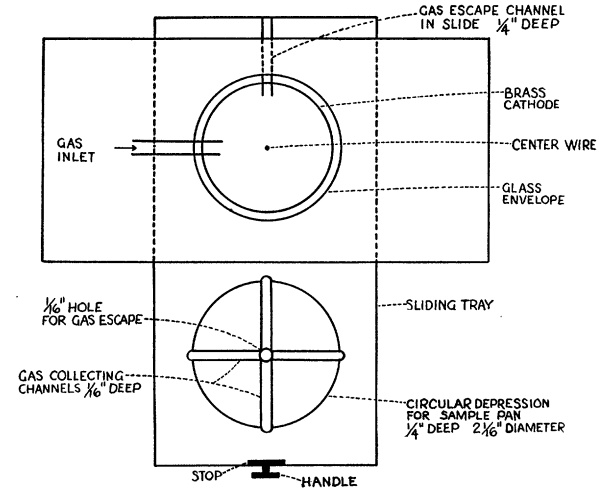


FIG. 2. Top view.

substances may also be spread out in this manner. The sample so prepared may then be inserted in the sliding tray and pushed into the counter.

A simplified version of the Geiger tube can be made omitting the sliding arrangement for insertion of the sample and including a side arm for a gas outlet. In this case the sample is placed on the copper disk as usual, the copper disk on a flat brass plate, and the counter over the sample. The tube can be made relatively airtight by pressing down on the glass tube or by using stopcock grease or wax at the junction of the glass tube and brass plate. Since the counting gas is always under a slight positive pressure, small leaks are of no impor-

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