

exposure of 20,000 to 25,000 equivalent roentgens to the first mm of tissue. A few weeks after radiation, the quantity of acid secreted had decreased to levels ranging from an acidity to 10% of the quantity secreted before radiation. The longest period of observation after radiation in any animal was 125 days.

There were no changes in blood count, weight, or general condition of the animals which could be attributed to the radiation.

In summary, the gastric mucosa of Heidenhain pouches of five dogs was irradiated with beta particles of P^{32} , with a resultant marked decrease in the quantity of acid in the pouch secretion.

If similar techniques in humans are contemplated it is important to note that the dog Hepi had return of acidity in its Heidenhain pouch 3 months after irradiation. When the animal was sacrificed two weeks later, autopsy showed an ulcer of the pouch mucosa at the site of previous biopsy.

References

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β -Glucuronidase Activity

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During the past few months, a method has evolved which gives promise as a diagnostic aid in female genital carcinoma. Fishman (1) first reported an increased activity of the enzyme β -glucuronidase in carcinoma tissue (breast, esophagus, stomach, and colon). Subsequently (2), results from other malignant tissues were added to his series. The possibility of measuring the tissue activity of β -glucuronidase from the more accessible pelvic malignancies (vulva, vagina, and cervix) as a diagnostic aid is self-evident. Furthermore, if present indications prove correct, the vaginal fluid which bathes the cervix and vagina should become a rich source of this enzyme in the presence of a lower genital tract malignancy.

Accordingly, β -glucuronidase assays were made on the various genital tissues and on vaginal fluid for the purpose of establishing the range of activity in histologically benign lesions, and in carcinoma. Tissues were weighed, homogenized in water, and the centrifuged homogenates assayed, using phenolphthalein glucuronide as substrate, according to the method described by Fishman, Springer, and Brunetti (3). Using a pipette, 0.1-ml portions of vaginal fluid were suspended in 3 ml of distilled water or Tyrode's solution. Assays were made on both uncentrifuged specimens and on the centrifuged supernatants. Values were expressed as γ of phenolphthalein liberated per g of tissue or ml of vaginal fluid per hr. Our results are tabulated.

In the absence of pregnancy, the range of β -glucuronidase activity in those cervixes without histological evidence of malignancy was from 23 to 330 γ of phenolphthalein per g tissue per hr (Table 1). The upper limit of this group was significantly less than the lowest value for cervical carcinoma, 543 γ , #299510 (Table 2).

TABLE 1
GLUCURONIDASE ACTIVITY

Tissue	Cases	γ of β -Glucuronidase*	
		Range	Average
Benign cervix	13	23- 330	142
Pregnant cervix	8	221- 591	384
Malignant cervix	6	543-2790	1274

* β -Glucuronidase expressed as γ phenolphthalein liberated per g of tissue per hr.

A variety of clinical and pathologic diagnoses were found among the benign cervixes studied. Most were from cervical biopsies obtained to rule out malignancy. Others included senile vaginitis, cervical erosion, acute and chronic cervicitis, and some apparently normal organs. A histologic examination of the tissue adjacent to the area assayed established an absence of malignancy. The activity of β -glucuronidase in vaginal mucosa (non-pregnant) was similarly low. The enzyme activity of pregnant cervix (at term) was higher, and there was some

TABLE 2
GLUCURONIDASE ACTIVITY OF UNTREATED
CARCINOMA TISSUE

Identification	Microscopic diagnosis†	γ of β -Glucuronidase‡
436773	Sq. ca. cx.	934
438049	Sq. ca. cx.	2790
159341	Sq. ca. cx.	1634
452839	Sq. ca. cx.	847
299510	Sq. ca. cx.	543
45424	Sq. ca. cx.	897
A.M.*	Sq. ca. vag.	688
A.S.*	Sq. ca. vag.	680

* Patient at another hospital.

† Sq. ca. cx.—Squamous carcinoma of cervix; Sq. ca. vag.—squamous carcinoma of vagina.

‡ β -Glucuronidase expressed as γ phenolphthalein liberated per g of tissue per hr.

overlapping with the malignant group. No explanation is offered at the present time for this finding.

The malignant tissues studied are tabulated in Table 2. The range of activity was high. Two specimens of endometrial carcinoma were assayed. These measured 11,930 and 6,370 γ . The lower of these values is within the range of glucuronidase activity for endometrium in women with normal menstrual periods (895 to 9040 γ) (4) and both are well within the range for endometrium in patients with functional uterine bleeding (1,180 to 20,050 γ) (5). The activity of β -glucuronidase in normal ovary and in benign ovarian tumors varied greatly. It

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).

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.

trifuged (Table 3). It is apparent from studies on vaginal secretion obtained from women with benign lesions that false positive tests occurred (Table 4). These

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.

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.

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The Nature of the Pigmented Sheath in *Drosophila* Tumors¹

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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, tumorous 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

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