

Beta-Glucuronidase Activity in Human Female Genital Cancer

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In 1937 Marrian (1) described a reaction whereby estriol glucuronide was hydrolyzed during its passage through mouse intestine. This action was ascribed to a β -glucuronidase.¹ In a series of experiments, Fishman (2) and others (3) associated the activity of this enzyme more closely with the metabolism of glucuronic acid. The glucuronidase activity of mouse liver was increased following the administration of borneol and menthol, which are conjugated in animals as glucuronides, while injections of estrogen increased the glucuronidase activity of mouse uteri. The concept of "metabolic conjugation" rather than detoxification was applied to β -glucuronidase activity in mouse uteri where it was suggested the conjugated estriol glucuronide was possibly used by the cells (4). Recently, Odell and Fishman (5) described cyclic changes of glucuronidase activity in normal human endometrium. These changes closely parallel the reported titres of blood and urinary estrogens during the menstrual cycle. In addition blood and urine glucuronidase activity were found increased during pregnancy (6, 7). Moreover, the fall in serum glucuronidase activity postpartum was delayed by the administration of estrogens, notably stilbestrol (8).

A close association with cellular activity led to the study of this enzyme in cancer tissue. Fishman (9, 10) noted greater activity of glucuronidase in breast carcinomas and involved lymph nodes, and in gastric, bowel, and metastatic carcinomas, than in normal uninvolved tissues. Moreover, an inhibitor substance (11) low in cancer tissue was detected. Preliminary studies on female genital cancer have been reported by Odell and Burt (12).

EXPERIMENTAL

The method used was that described by Fishman, Springer and Brunetti (13). Fresh tissues² were weighed,

¹ β , since glucuronic acid occurs only in the beta position in nature.

² Histologic preparations were made from tissues adjoining those assayed. Actively growing, non-necrotic tumor tissue was selected.

homogenized with distilled water, centrifuged and the supernatant fluids assayed for β -glucuronidase activity. Findings are summarized in Tables 1, 2, 3, and 4. Except for the pregnant cervix, a consistent difference in glucuronidase activity occurred between histologically proven cases of squamous celled cervical carcinoma (which were high) and histologically non-malignant cervixes (which were low). Glucuronidase values for endometrial carcinoma, however, were within the range of non-malignant endometrium. Decidua had its greatest activity in early pregnancy; values over 1000 γ per gm. per hour of incubation being obtained from patients under 12 weeks pregnant. It is of interest that the activity in non-malignant and malignant endometrium and decidua are much greater than in any other genital tissue, and some of the highest obtained in the body. Four cases of primary squamous celled carcinoma of the vagina and two of the vulva were studied. In five of these β -glucuronidase activity was increased. A radical vulvectomy was performed on one patient with vulvar carcinoma. The glucuronidase activity of the vulvar lesion and a histologically involved lymph node were high and comparable.

As to the non-malignant cervix histological study revealed a variety of diagnoses. Among these, in addition to histologically normal cervix, were acute and chronic cervicitis with Nabothian cysts, cervical erosion, and leukoplakia. No correlation could be found between the types of benign lesion and the activity of β -glucuronidase in the tissue. Malignant cervical tissues, however, were greater in the proliferative (exophytic) growths than in ulcerative (endophytic) types. As to the non-malignant endometrium histological study revealed all degrees of normal, hypoplastic and hyperplastic growth. Glucuronidase activity was greatest in endometrial hyperplasia. All endometrial tissues were obtained incident to hysterectomy or at the time of diagnostic curettage. The range of β -glucuronidase activity in these specimens was greater (upward) than that reported for endometrium from women with normal menstrual cycles (5).

Serial determinations showed a progressive increase in glucuronidase activity from the portio of the cervix to the endometrium, but little difference between various sites of endometrium (Table 3). Irradiation caused a decrease in tissue β -glucuronidase activity (Table 4). A cervix which was histologically involved with cancer was higher in activity than uninvolved portions. As

such, these data on tissues indicate a significant difference between the glucuronidase activity of non-malignant (non-pregnant) and malignant cervix, no difference between non-malignant and malignant endometrium, and marginal differences in vulva and vagina.

In the light of these findings, one would expect the vaginal fluid, which bathes the cervix and vagina, to become a rich source of this enzyme in the presence of

Untreated cervical cancer was associated in every case with a high activity (over 320 γ per ml. vaginal fluid per hour) of enzyme in the uncentrifuged vaginal fluid suspension. Following treatment this activity decreased as evidenced by the collective data³ (Fig. 1) and by the changes observed in individual cases (Table 4). Care must be employed to avoid contamination of the vaginal fluid with lubricating jelly and fresh blood. Likewise,

TABLE 1
 β -GLUCURONIDASE ACTIVITY IN UNTREATED GENITAL CANCER

DIAGNOSIS	NO. OF CASES	γ OF β -GLUCURONIDASE ACTIVITY								
		Tissue		Vaginal Fluid†						
		Max.	Min.	Mean*	Uncentrifuged		Centrifuged			
				Max.	Min.	Mean*	Max.	Min.	Mean*	
Squamous cell carcinoma of cervix	18	4200	512	1641	1930	320	1020	1300	62	467
Adenocarcinoma of fundus	8	11930	1352	6774	1218	480	835	493	16	227
Squamous carcinoma of vagina	4	1440	680	891	976		976			
Squamous carcinoma of vulva	2	925	237	581						

Figures express γ of phenolphthalein liberated per gm. of tissue or ml. of vaginal fluid per hour of incubation. Only untreated cases are included.
* Arithmetic mean.
† Vaginal fluid was available for assay on 14 cervical and 5 fundal carcinomas, and 1 vaginal carcinoma.

genital malignancy. Using an immunologic pipette 0.1 ml. portions of vaginal fluid were suspended in 3 ml. of distilled water. Assays were made on both uncentrifuged specimens and on supernatants (Fig. 1). In clinically benign conditions, there was a wide distribution of values in uncentrifuged specimens. If 300 γ per ml. vaginal

douches previous to examination are discouraged. If added *in vitro* venous blood will inhibit the activity of glucuronidase in vaginal fluid. This inhibition, however, is not always observed when the bleeding originates from the corpus, possibly because of the high activity of glucuronidase in malignant and benign endometrium. Of the two methods, tissue *versus* vaginal fluid assay, the latter (vaginal fluid) is easier and more practical for large surveys.

TABLE 2
FREQUENCY TABLE OF β -GLUCURONIDASE ACTIVITY IN BENIGN TISSUES

γ OF ACTIVITY	CERVIX		ENDOMETRIUM		Vul-va	Va-gina	Ovary
	Non-preg-nant	Preg-nant	Non-preg-nant	Preg-nant			
1- 100	16	3				1	1
101- 200	16	1			1	2	1
201- 300	6	5					
301- 400	3	3		1	1	2	2
401- 500		2		1			4
501- 600		2		1			1
601- 700		1		1	1		
701- 800		1			1		1
801- 1000				2			
1001- 3000			3	1			1
2001-10000			31	3			
10000-20000			5	1			
Over 20,000			3				

Figures express γ of phenolphthalein liberated per gm. of tissue per hour of incubation. Figures for additional benign tissues were: Corpus luteum 2218, 953, 1820; dermoid 2530; pseudomucinous cystadenoma 187,338; fibromyomata 0, 3, 82, 402; endometrial polyp 4360, 1990, 1052; fallopian tube 384, 402, 142; myometrium 319, 173, 607, 551.

fluid per hour of incubation is used as a practical line of demarcation between a positive and negative test, the per cent of false positives was 18. Uncentrifuged specimens were higher than centrifuged supernatants (Fig. 1), thus localizing some of the activity of the enzyme in the solid component of the suspension. False positive tests (above 300 γ) were found during pregnancy, in menstrual blood, and in vaginitis. The latter observation suggests bacterial action but does not explain the close correlation with tissue activity in cancer patients.

TABLE 3
SERIAL DETERMINATIONS OF β -GLUCURONIDASE ACTIVITY

Identification	Cervix	Endo-cervix	Internal os	Endome-trium	
				(mid-portion)	(fundus)
408607	126	248			10100
81911	48	38	92	4760	3450
435151	118	136	1520	4180	8160
377895	106	178	365	8040	7090
450707	55	95			
*460805	133	143		993	

Figures express γ of phenolphthalein liberated per gm. of tissue per hour of incubation.
* Porro section.

DISCUSSION

It has been suggested that the activity of β -glucuronidase is related to cellular proliferation (14). It seems pertinent that its activity is higher in those normal genital organs (endometrium and corpus luteum) in which mitotic activity is usually present. In addition, the activity of β -glucuronidase has been closely associated with glucuronide formation (2, 3), notably with estrogen (4).

Since the range of activity of β -glucuronidase in malignant tissue is increased over the non-malignant in cervix but not in endometrium, the role of

³ The collective data included all treated cases of genital cancer (cervix, uterus, and vulva).

this enzyme in cancer tissue becomes a point of interest. If glucuronidase is primarily concerned with the conjugation of estrogen, is endometrial carcinoma different from non-malignant endometrium in its metabolic requirement for steroid?

In fact, the outstanding feature of an increased activity of β -glucuronidase in genital cancer, in non-malignant endometrium, in corpus luteum, and in pregnancy (6, 7) has been its close association with cellular growth.

TABLE 4

EFFECT OF IRRADIATION ON β -GLUCURONIDASE ACTIVITY

Identification	Age	Group	Tissue	Vaginal fluid		Date	Remarks
				U	C		
460648	56	II	1308	1860	1300	1-20-49	
				540	113	2-5-49	4572 mg. hr.
				275	176	2-18-49	1560 mg. hr.
				268	100	2-27-49	
				134	107	3-11-49	
460099	62	III	398 1537	808	136	1-29-49	Bloody fluid
				900	197	2-5-49	4560 mg. hr.
				329		2-21-49	1560 mg. hr.
				480	346	3-2-49	1560 mg. hr.
				530	365	3-18-49	
462123*	67		877, 239 1352 798	231	133	2-9-49	
				530	365	2-27-49	4560 mg. hr.
				231	133	3-14-49	

The table includes irradiation treatment in mg. hours of radium and pertinent notes. Figures express γ of phenolphthalein liberated per gm. of tissue or ml. of vaginal fluid per hour of incubation.

Group—League of Nations Classification; U—Uncentrifuged suspensions of vaginal fluid and water; C—The supernatant fluid after centrifugation.

* Adenocarcinoma of fundus with cervical extension.

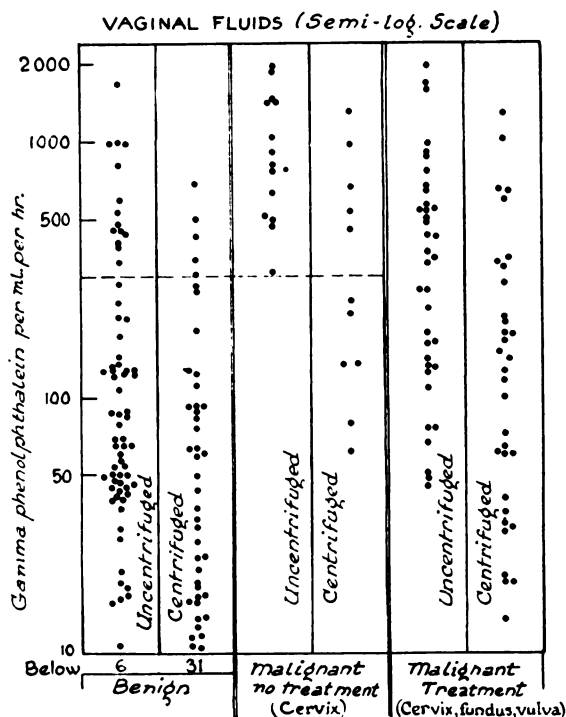


FIG. 1.—Illustrates γ of phenolphthalein liberated per ml. vaginal fluid per hour of incubation.

It would not seem to be likely. If glucuronidase is related to cellular growth, the similarity between non-malignant and malignant endometrium, together with the increased activity in cervical, vaginal and vulvar cancer, is understandable, since all of these are characterized by mitotic figures.

Micro-Kjeldahl determinations were made on homogenized tissues for total protein. Some direct relationship was observed between the total protein of the homogenate and the activity of the enzyme (15). The interpretation of this observation would include the possibility that the activity of β -glucuronidase is related to the cellular structure of the tissue assayed.

SUMMARY

As to tissues, the activity of the enzyme β -glucuronidase is greater in malignant cervix than in non-malignant cervix. In adenocarcinoma of the uterus, however, glucuronidase activity does not differ from that found in endometrium from women with benign uterine bleeding. Increased activity of this enzyme is present in some pregnant cervixes.

As to vaginal fluid, assays on the uncentrifuged suspensions showed consistently high values in the presence of untreated cervical cancer. In the presence of clinically benign conditions, false positive tests occurred in 18 per cent of the material studied.

Following irradiation treatment for cervical cancer, the activity of β -glucuronidase in the tissue and in the vaginal fluid declined.

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