A Study on the Aetiological Factors of Bilharzial Bladder Cancer in Egypt. 3. Urinary β-Glucuronidase

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Abstract—*Human tissues and* E. coli *β-glucuronidase showed distinct differences in their properties with respect to substrate specificity, pH optima and effect of different agents.*

E. coli *and pseudomonas sp. the most commonly occurring bacteria in the urine of bilharzial and bladder cancer patients showed markedly high levels of β-glucuronidase.*

Increased level of β-glucuronidase activity had been found in the urine of bilharzial and bladder cancer patients compared to that of normal urine. β-glucuronidase in urine showed two peaks of activity one was found to be of tissue origin and the other of bacterial origin, with optimal activity at pH 5.0 *and* 7.0 *respectively. A positive correlation was clearly demonstrated between type and severity of bacterial infection and urinary β-glucuronidase level. The possible role of urinary β-glucuronidase in the aetiology of bilharzial bladder cancer had been discussed.*

INTRODUCTION

REPORTS indicated that bladder cancer was the most frequent cancer among Egyptians [1]. Its origin was linked to long-term bladder bilharziasis and the commonly associated alkaline pyogenic sepsis.

Bacterial infection of the urinary tract complicating bilharziasis was extremely common [2].

S. haematobium had been reported by many workers to cause elevated levels of B-glucuronidase in the urine of bilharzia infested patients [3–8]. A link has been indicated between an increased urinary excretion of B-glucuronidase and the development of cancer of the bladder [9, 10]. Among substrates for this enzyme likely to be present in urine are some potentially carcinogenic compounds excreted as glucuronides. There is no definite conclusion reported in literature concerning the origin of urinary β-glucuronidase. However high enzyme activity is usually present in liver, kidney, spleen [11, 12], cancer [13] lymphatic tissues [14, 15], leucocytes [16] and several types of bacteria [17–19].

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The aim of the present investigation is to characterize β-glucuronidase from different sources, to help identify the origin of urinary β-glucuronidase in normal subjects, bilharzial infested and bladder cancer patients and to evaluate its possible role in bladder carcinogenesis in bilharziasis.

MATERIALS AND METHODS

Tissue specimens

Human liver, spleen, kidney and normal bladder mucosa were obtained from post-mortem, while the cancer bladder tissue was obtained after cystectomy. Tissue homogenates for enzyme assay were prepared using distilled water.

Preparation of leucocytes

The leucocytes were prepared from blood samples obtained from blood donors according to the method of Peacock *et al.* [20].

Bacterial investigations

Viable bacterial counts were carried out immediately after collection using the surface spreading method and a standard platinum

4 mm loop that holds 0.01 ml [21]. The bacterial species in each urine sample were identified and isolated in pure strains [22–24].

Enzyme assay in tissue homogenate and bacteria

The activity of β-glucuronidase was determined by the method of Fishman [25] after modification using *p*-nitrophenyl-β-glucuronide or phenolphthalein-β-D-glucuronide (Sigma Chemical Co.) as substrates. Assay mixture of 0.5 ml final volume containing 2.5 mM substrate and 60 mM citrate–phosphate buffer adjusted to the required pH, unless it has been otherwise stated, was incubated at 37°C for the appropriate time. The reaction was stopped by the addition of 2.5 ml, 0.05 N NaOH. The optical density was measured at 405 nm in case of *p*-nitrophenol and at 535 nm in case of phenolphthalein, in cuvettes with 1.0 cm light path using Unicam Model SP 500 spectrophotometer. A unit of enzyme is defined as that amount releasing 1 μg *p*-nitrophenol or 1 μg phenolphthalein/hr/mg protein.

Enzyme assay in urine

β-Glucuronidase activity was assayed in the fresh morning urine samples immediately after collection. Enzyme assay was carried in a final volume of 0.5 ml containing 2.5 mM *p*-nitrophenyl glucuronide (Sigma Co. Ltd.) and 0.4 ml of urine adjusted to pH 5.0 or 7.0 using concentrated HCl or NaOH to avoid dilution. Urinary β-glucuronidase activity is expressed as μg *p*-nitrophenol released per hr/mg creatinine or per millilitre urine.

Determination of creatinine

Creatinine was estimated using the alkaline picrate method [26].

Determination of protein

Protein estimation of tissue preparations had been carried out according to Lowry's method [27].

RESULTS

Level of B-glucuronidase activity in different tissue homogenates and bacteria

As shown in Table 1, when *p*-nitrophenyl glucuronide was used as a substrate liver and bladder tumour showed a markedly high enzyme level (18.4 and 18.6 unit respectively) when compared with normal bladder mucosa, kidney, spleen and leucocytes (6.2, 10.5, 6.4 and 4.4 units respectively). On the other hand intact *E. coli* showed the highest value (27.2 units). Intact *E. coli* was used to simulate the *in vivo* conditions.

Taking the activity obtained with *p*-nitrophenyl glucuronide as 100%, the activity using phenolphthalein glucuronide was found to be 120% for leucocytes and 148–200% for normal tissue homogenates and 360% in case of bladder tumour. Meanwhile, it is apparent from Table 1 that phenolphthalein glucuronide is not the proper substrate for intact bacteria.

Effect of buffers

The effect of different buffers on activity level of tissues and bacterial β-glucuronidase is shown in Table 2 where the activity obtained in presence of citrate–phosphate buffers is taken as 100%. Human tissue β-glucuronidase in all buffers shows 50–180% increase in activity above that obtained with citrate-phosphate. On the other hand bacterial β-glucuronidase shows maximum ac-

Table 1. Substrate specificity of tissue and bacterial β-glucuronidase

	Enzyme source						
Substrate	Liver	Kidney	Spleen	Leucocytes	Normal bladder	Bladder tumour	Bacteria (*E. coli*)
p-Nitrophenyl glucuronide	100 (18.43)	100 (10.51)	100 (6.43)	100 (4.39)	100 (6.19)	100 (18.67)	100 (27.21)
Phenolphthalein glucuronide	177	180	200	120	148	360	0

( ) absolute activity expressed as μg *p*-NP or μg ph.ph./hr/mg protein.
Results expressed as per cent taking activity using *p*-NPG as 100.
Data are an average of three experiments, each experiment run in triplicate.
Citrate–phosphate buffer used (60 mM final concentration).
All activity measured at pH 5.0 except that of bacteria at pH 7.0.
Bladder tumour was of squamous cell carcinoma type.

Table 2. Effect of buffer type on tissues and bacterial β-glucuronidase

Buffer	Liver	Kidney	Spleen	Leucocytes	Normal bladder	Bladder tumour	Bacteria (*E. coli*)
Citrate–phosphate (0.1 M)	100 (18.43)	100 (10.51)	100 (6.43)	100 (4.39)	100 (6.19)	100 (18.67)	100 (27.21)
Acetate (0.1 M)	233.3	235.5	250.0	221.4	181.9	145.2	—
Veronal–acetate (0.1 M)	283.0	285.1	227.0	148.0	257.0	307.0	10.0
Sodium cacodylate (0.06 M)	230.0	250.0	180.0	210.0	155.0	200.0	13.0
Dimethyl glutarate (0.06 M)	230.0	235.0	220.0	315.0	200.0	285.0	70.0
Tris–maleate (0.1 M)	230.0	235.0	240.0	263.0	212.0	233.0	85.0
Phosphate (0.1 M)	100.0	100.0	112.5	171.0	120.0	100.0	80.0

( ) absolute value of activity expressed as μg *p*-NP/hr/mg protein.
Results expressed as per cent taking activity in presence of citrate-phosphate buffer as 100%.
Activity measured at pH 5.0 except that of bacteria at pH 7.0.

tivity in citrate–phosphate buffer, while other buffers showed varying degrees of inhibition ranging from 10 to 90%.

Effect of pH on enzyme activity

The pH activity curves of β-glucuronidase of different tissues and *E. coli* in citrate–phosphate buffer are shown in Fig. 1. Tissue β-glucuronidase display similar patterns, with an optimum activity at pH 5.0. When acetate buffer was used (not illustrated) the optimal enzyme activity was shifted to 4.4–4.6 accompanied with an increase in activity amounting to double that obtained with citrate–phosphate buffer. Bacterial β-glucuronidase shows maximal activity at pH 7.0.

Effect of urine constituents on enzyme activity

The effect of some urine consittuents on β-glucuronidase activity of human tissues and bacteria is shown in Table 3. Tissue β-glucuronidase is not inhibited by urea, cysteine, pyrophosphate, oxalate, nitrite, nitrate or creatinine, whereas bacterial β-glucuronidase was inhibited by 67–97% in the presence of these agents. Urine adjusted to the required pH inhibits tissue enzyme by 40–52% and bacterial enzyme by 60%.

Albumin (0.01%) activates tissue β-glucuronidase by 25–40% whereas it inhibits bacterial enzyme by 30%. DNA (0.03%) inhibits tissue enzyme by 10–15% and bacterial enzyme by 30%.

Enzyme activity level in different strains of bacteria

β-Glucuronidase levels of different bacteria isolated from urine of bilharzial or bladder

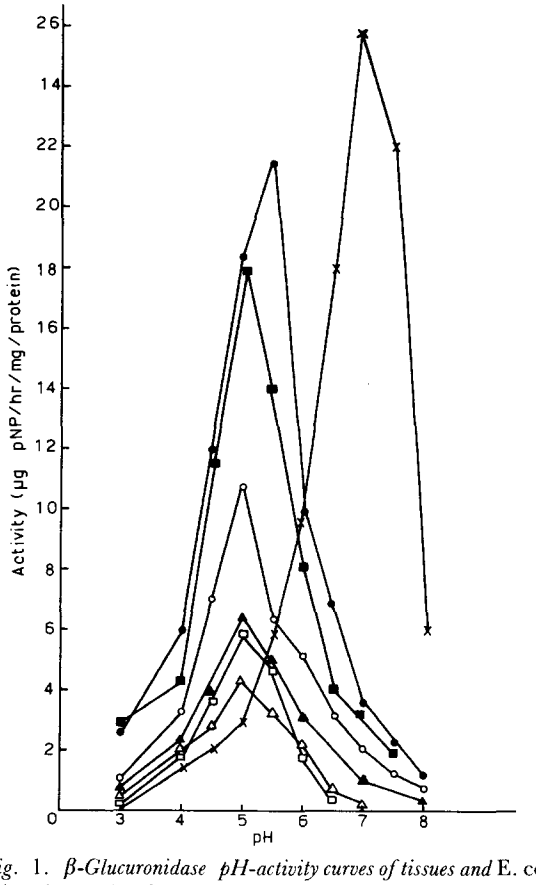

Fig. 1. β-Glucuronidase pH-activity curves of tissues and E. coli *using citrate phosphate buffer.* ×—× E. coli; ■—■ *bladder tumour;* ○—○ *kidney;* □—□ *normal bladder;* ●—● *liver;* △—△ *spleen;* ▲—▲ *leucocyte.*

Table 3. Effect of urine and different urinary constituents on tissues and bacterial β-glucuronidase

Agent	Liver	Kidney	Spleen	Leucocytes	Normal bladder	Bladder tumour	Bacteria (*E. coli*)
None	100 (18.43)	100 (10.51)	100 (6.43)	100 (4.39)	100 (6.19)	100 (18.67)	100 (27.21)
Urine	73.0	75.0	77.0	71.4	66.6	61.7	43.0
Urea (0.1 M)	93.0	100.0	91.0	100.0	85.0	91.0	15.0
Cysteine (0.2 M)	100.0	100.0	100.0	100.0	100.0	100.0	23.0
Sodium pyrophosphate (0.05 M)	100.0	100.0	100.0	100.0	100.0	85.0	5.5
Sodium oxalate (0.05 M)	100.0	100.0	100.0	125.0	100.0	100.0	33.0
Sodium nitrite (0.2 M)	100.0	100.0	100.0	95.0	100.0	83.0	5.5
Sodium nitrate (0.2 M)	100.0	100.0	100.0	125.0	100.0	100.0	11.0
Creatinine (0.2 M)	76.0	80.0	85.0	75.0	86.5	70.0	10.0
Albumin (0.01%)	142.0	125.0	140.0	135.0	100.0	142.0	70.0
DNA (0.03%)	85.0	100.0	95.0	88.0	90.0	85.0	74.0

( ) absolute value of activity expressed as μg *p*-NP/hr/mg protein.
Results expressed as per cent taking activity in absence of agent as 100%.
Enzyme assayed at pH 5 for tissue and pH 7.0 in case of bacteria, using citrate-phosphate buffer.

Table 4. β-Glucuronidase activity in bacteria isolated from the urine of bilharzial and bladder cancer patients

Type of bacteria	Incubation time (hr)	Enzyme activity: μg *p*-Nitrophenol/ hr/mg protein	Enzyme activity: μg *p*-Nitrophenol/ hr/10^6 bacteria
E. coli	3.5	27.21	17.14
Staphylococci	1.0	30.48	2.22
Pseudomonas sp.	8.0	9.51	7.50
Anthracoids	15.0	4.14	2.42
Salmonella sp.	18.0	3.39	3.57
Diphtheroids	18.0	1.98	2.32
Proteus sp.	30.0	0.30	0.10
Klebsiella sp.	30.0	0.17	0.05
Strep. faecalis	30.0	0.00	0.00

Results are an average of 3 experiments each experiment run in duplicate.

cancer patients are shown in Table 4. When the enzyme activity was calculated per mg protein of intact bacteria, *E. coli* and staphylococci showed the highest activity (27.2, 30.5 units) compared to proteus, klebsiella and *Streptococcus faecalis* (0.3, 0.2, 0.0 units). However when activity is calculated per 1 $\times 10^6$ bacteria, *E. coli* showed the highest activity (17.1 units). No activity was detected when phenolphthalein glucuronide was used as substrate even after 30 hr of incubation.

Effect of incubation time on enzyme activity

Figure 2 shows the effect of incubation time on liver and bacterial (*E. coli*) β-glucuronidase using citrate-phosphate buffer at pH 5.0 and 7.0 respectively. Liver β-glucuronidase shows a steady increase in activity with time, while there was a lag period in case of bacteria.

The effect of incubation time and bacterial counts on β-glucuronidase activity is shown in Fig. 3. With high bacterial counts (500,000)

enzyme activity is almost apparent after one hour of incubation and exhibits a steady increase. While in the presence of low bacterial counts (10,000) the enzyme activity takes a period of 3–5 hr of incubation before it becomes manifested.

Urinary β-glucuronidase

Figure 4 demostrates the level of urinary B-glucuronidase activity at different pHs the activity was expressed as μg *p*-nitrophenol/hr/mg creatinine of urine. Normal urine after 3 hr incubation showed a low peak of activity (1.6) at pH 5.0 while in bilharzial infested urine after the same incubation time two peaks were observed at pH 5.0 (4.3) and at pH 7.0 (1.6). Bladder cancer urine showed very high activity at pH 7.0 (19.8) and at pH 5.0 (12.0) after only 1–2 hr of incubation.

The effect of incubation time on β-glucuronidase activity, expressed as μg *p*-nitrophenol/hr/mg creatinine, of bilharzial urine is shown in Fig. 5. The activity at pH 5.0 after incubation for 4 hr did not show a

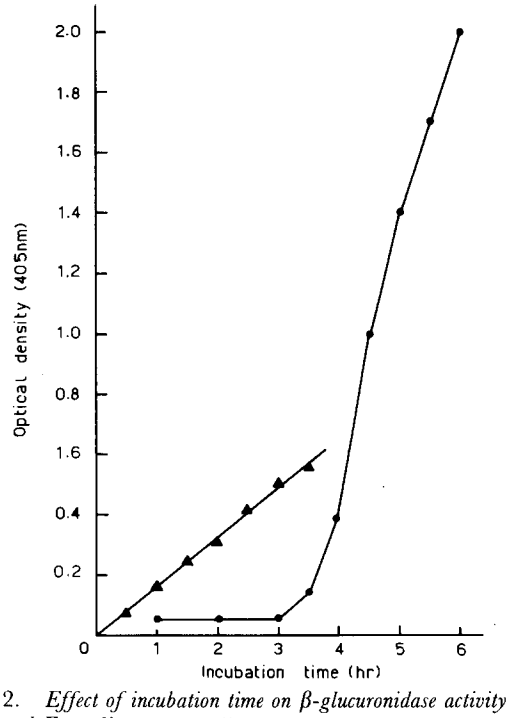

Fig. 2. Effect of incubation time on β-glucuronidase activity of liver and E. coli. ▲—▲ *liver;* ○—○ E. coli (100,000 *organisms*).

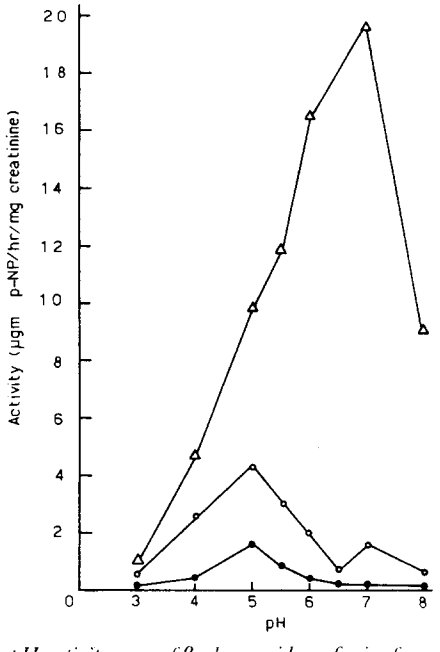

Fig. 4. pH-activity curve of β-glucuronidase of urine from normal, bilharzial infested and bladder cancer patients. ●—● *normal urine;* ○—○ *bilharzial urine;* △—△ *bladder cancer urine.*

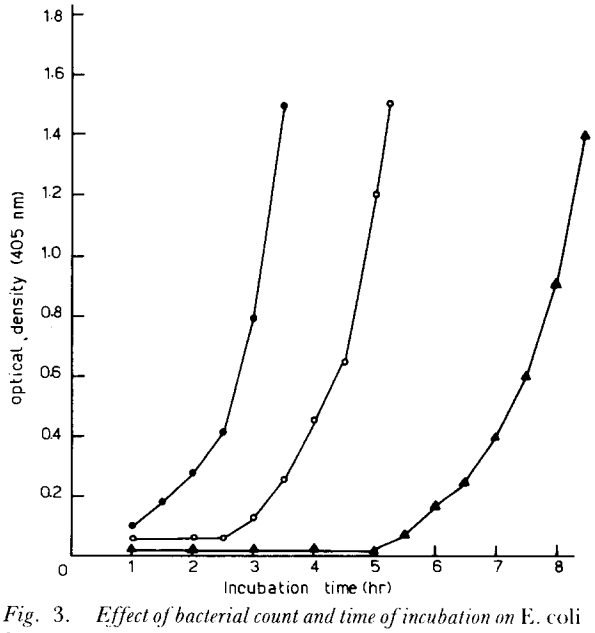

Fig. 3. Effect of bacterial count and time of incubation on E. coli *β-glucuronidase.* ▲—▲ 10,000 *organisms;* ○—○ 100,000 *organisms;* ●—● 500,000 *organisms.*

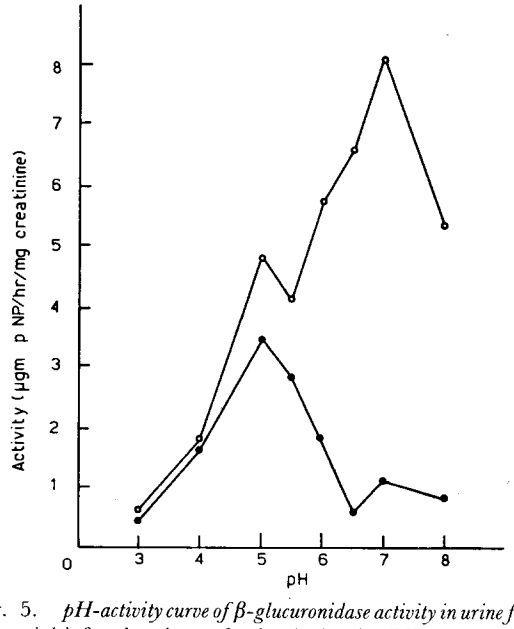

Fig. 5. pH-activity curve of β-glucuronidase activity in urine from bilharzial infested patients after incubation for different periods of time. ●—● *4 hr;* ○—○ *8 hr.*

marked difference from that obtained after 8 hr. Meanwhile, at pH 7.0 an abnormal increase after 8 hr of incubation amounting to 8 fold that observed after 4 hr was obtained. Accordingly, and due to the lag period before the manifestation of the bacterial activity at pH 7.0 incubation periods of 6–8 hr have been used, which also correspond to the period for which urine can be retained in the bladder.

Tables 5, 6 and 7 include data on urine of normal subjects, bilharzial infested and bladder cancer patients showing the bacterial species and their count per ml urine, pH and creatinine content of the urine and the pathological diagnosis of the bladder cancer together with the level of β-glucuronidase activity at pH 5.0 and 7.0.

Table 8 shows the incidence rates of the different bacterial types in bilharzial and bladder cancer urine. The most common organisms identified were *E. coli* (50% in bilharzial urine, 69.2% in bladder cancer urine), Pseudomonas sp. (13.6% in bilharzial urine and 19.2% in bladder cancer urine), Proteus sp., Klebsiella sp., anthracoids and diphtheroids. Streptococci and Staphylococci were often associated with any one of the previous organisms. Salmonella organisms were detected only in bilharzial urine.

Table 9 demonstrates the effect of 24 hr storage of urine samples without bacteriostatic agent on the activity of urinary β-glucuronidase. Activity is markedly increased by storage particularly at pH 7.0 (40 fold) parallel to the increase of bacterial count (25 fold).

The activity level of β-glucuronidase in normal bladder mucosa was found to be (6.19), with a remarkable increase in all pathological types of bladder carcinoma (transitional cell carcinoma 9.57, squamous cell carcinoma 11.46 and adenocarcinoma 18.67).

DISCUSSION

β-Glucuronidase has been studied by many workers in view of its importance in releasing free carcinogens [9, 10]. Abdel Tawab *et al.* [28] observed increased enzyme activity at pH 7.0 in the urine of bilharzia infested patients which is markedly decreased after antibiotic therapy. However, many workers overlooked the measurement of urinary β-glucuronidase at pH 7.0, the optimal pH of the enzyme of bacterial origin [28].

Distinguishing properties characterizing human tissue β-glucuronidase from *E. coli* β-glucuronidase were obtained. The hydrolysing capacities of the tissue enzyme and the bacterial enzyme are conspicuously different.

The failure of intact bacteria to utilize phenolphthalein glucuronide, may be ascribed to the impermeability of the bacterial cell wall to that particular substrate. This point of view is supported by Jacox [18] who reported a 5–10 fold increase in bacterial enzyme activity after sonication. The high affinity of bacterial β-glucuronidase to *p*-nitrophenyl-glucuronide agreed with the results reported by other investigators [29, 30].

Tissue and bacterial enzymes show two different and distinct pH optima. Different pH optima were reported in literature for the bacterial and tissue enzyme depending on the purity of the enzyme, the buffer type and the substrate used [18, 29].

Bacterial and tissue β-glucuronidase showed remarkable differences with respect to the effect of some urine constituents on the enzyme activity. However, when whole urine was added to the incubation medium it inhibited 40% of the tissue enzyme and 60% of the bacterial enzyme. A wide variety of organic substances of high or low molecular weights, have been shown to activate, inhibit or inactivate β-glucuronidase [12, 31, 32].

E. coli and pseudomonas, commonly present in the bladder of bilharzial patients, showed a remarkably high level of β-glucuronidase. Many workers reported β-glucuronidase activity in bacteria [33, 34]. However, not all bacteria possess β-glucuronidase [35–37].

The lag period observed before the β-glucuronidase enzyme activity of bacteria became detectable and its correlation to the bacterial count might be due to the period necessary for the substrate to be available to the enzyme, or to the period necessary for the induction of enzyme synthesis. Stimulation of *E. coli* to produce β-glucuronidase after the addition of β-glucuronide in the medium had been reported by many investigators [18, 36, 38]. Based on data presented here it is evident that one can distinguish biochemically between bacterial and tissue β-glucuronidase but not between the enzyme from different tissue sources.

The addition of bacteriostatic agents during the collection of 24 hr urine samples appears to be a critical factor. Melicow *et al.* [10] claimed that the bacteriostatic agent was not essential. However, their experiments yielded false increase in the enzyme activity which appears to be due to bacterial multiplication.

Most investigators used phenolphthalein glucuronide as substrate for their urinary en-

Table 5. *Urine samples of normal subjects*

Case No.	Age	Sex	pH	Creatinine g/l	Bacteria Type	Bacteria Count per ml $\times 10^3$	β-Glucuronidase activity pH 5.0 per mg creatinine	pH 5.0 per ml urine	pH 7.0 per mg creatinine	pH 7.0 per ml urine
1	75	M	4.8	1.15	*E. coli*	0.4	0.68	0.70	0.00	0.00
2	40	M	5.5	1.30	*E. coli*	0.5	1.08	1.70	0.25	0.40
3	23	M	6.2	1.80	*E. coli*	0.5	0.10	0.20	0.22	0.40
4	24	M	5.0	1.40	*E. coli*	0.6	1.56	1.35	0.20	0.50
5	23	F	5.2	0.85	*E. coli*	2.0	2.74	2.33	0.39	0.33
6	38	M	5.0	1.15	*E. coli*	6.0	1.50	1.30	0.10	0.50
7	25	F	4.6	0.53	*E. coli* + Diphtheroids	0.5	1.31	0.90	0.00	0.00
8	17	F	5.0	1.20	*E. Coli* + Anthracoids	1.0	0.41	0.60	0.06	0.10
9	36	M	6.0	1.15	*E. coli* + Diphtheroids	1.8	1.82	2.10	0.60	0.70
10	40	F	5.7	1.20	*E. coli* + Streptococci	5.2	2.43	2.80	0.17	0.40
11	20	F	5.5	1.20	*E. coli* + Diphtheroids + Streptococci	18.0	2.83	3.40	0.08	0.10
12	24	F	4.5	1.30	*E. coli* + Staphylococci	20.0	1.02	1.60	0.32	0.50
13	39	F	5.5	1.40	Diphtheroids	1.0	1.35	1.90	0.35	0.50
14	22	F	5.0	1.00	Diphtheroids	0.4	1.50	2.20	0.00	0.00
15	22	F	5.0	0.73	Anthracoids	18.5	5.47	3.99	0.22	0.16

Table 6. *Urine samples of bilharzial infested patients*

Case No.	Age	Sex	pH	Creatinine g/l	Bacteria Type	Bacteria Count per ml $\times 10^3$	β-Glucuronidase activity pH 5.0 per mg creatinine	pH 5.0 per ml urine	pH 7.0 per mg creatinine	pH 7.0 per ml urine
1	17	M	5.0	1.20	*E. coli*	6.0	11.81	15.0	0.90	2.5
2	16	F	5.3	1.23	*E. coli*	7.0	1.79	2.2	0.48	0.6
3	16	M	6.2	0.30	*E. coli*	7.3	8.30	2.5	1.33	0.4
4	36	M	5.5	2.00	*E. coli*	18.4	1.85	3.7	1.50	3.0
5	16	M	6.0	0.44	*E. coli*	100.0	9.54	4.2	3.18	1.40
6	32	M	5.5	1.00	*E. coli* + Staphylococci	2.0	4.72	5.0	1.10	0.7
7	20	M	5.0	0.53	*E. coli* + Streptococci	3.8	5.28	1.1	0.94	0.3
8	25	M	5.5	1.15	*E. coli* + Diphtheroids	11.0	2.82	3.3	0.45	0.6
9	35	M	5.5	0.76	*E. coli* + Diphtheroids	22.0	3.02	2.3	2.36	1.8
10	40	M	5.0	0.92	*E. coli* + Diphtheroids	36.0	3.80	3.5	1.19	1.1
11	40	M	6.2	1.15	*E. coli* + Pseudomonas sp.	38.0	2.60	3.0	0.76	0.9
12	18	M	5.5	0.61	Salmonella sp.	0.6	7.66	4.6	0.83	0.5
13	16	M	6.0	1.30	Proteus sp.	1.5	3.41	4.1	0.00	0.0
14	32	M	5.0	0.69	Klebsiella sp.	1.5	8.98	6.2	1.59	1.1
15	33	M	6.0	0.84	Staphylococci	4.2	1.30	1.1	0.35	0.3
16	20	M	5.0	1.30	Streptococci	7.5	1.73	2.3	0.00	0.0
17	16	M	5.7	1.60	Salmonella sp.	7.8	1.87	3.0	0.56	0.9
18	22	M	5.5	1.07	Staphylococci	8.9	3.21	3.4	0.00	0.0
19	26	M	5.0	2.00	Strept. + Pseud. sp.	9.0	1.25	2.5	0.25	0.5
20	19	M	5.0	2.00	Staphyl. + Diphtheroids	12.0	2.75	5.5	0.55	1.1
21	14	F	5.2	1.00	Pseudomonas sp.	20.0	2.62	2.6	1.20	1.2
22	19	M	5.3	2.00	Klebsiella sp.	15.0	1.55	3.1	0.05	0.1

Table 7. Urine samples of bladder cancer patients

Case No.	Age	pH	Creatinine g/l	Bacteria Type	Bacteria Count/ml $\times 10^3$	Pathology	β-Glucuronidase activity pH 5.0 per mg creatinine	pH 5.0 per ml urine	pH 7.0 per mg creatinine	pH 7.0 per ml urine
1	45	5.5	0.53	*E. coli*	17.5	Squamous carcinoma	8.95	4.70	2.66	1.40
2	45	5.5	0.49	*E. coli*	95.0	Squamous carcinoma	0.40	0.20	6.70	3.30
3	42	5.5	0.30	*E. coli*	320.0	Squamous carcinoma	10.40	3.12	20.80	6.25
4	39	6.0	0.76	*E. coli*	3,000.0	Squamous carcinoma	21.97	16.50	49.90	37.50
5	50	6.5	0.29	*E. coli*	4,500.0	Squamous carcinoma	20.64	6.00	51.60	15.00
6	50	6.5	0.84	*E. coli*	250.0	Squamous carcinoma	3.60	1.70	6.25	4.00
7	40	7.5	0.30	*E. coli*	150,000.0	Squamous carcinoma	7.22	17.50	49.90	45.00
8	42	7.4	0.87	*E. coli*	500,000.0	Squamous carcinoma	70.58	60.00	235.29	200.00
9	54	6.0	0.52	*E. coli* + Staph.	18.3	Anaplastic carcinoma	6.00	3.00	1.80	0.90
10	54	7.2	0.46	*E. coli* + Strept.	56.0	Squamous carcinoma	6.34	2.91	12.49	5.74
11	49	5.6	0.47	*E. coli* + Staph.	89.0	Squamous carcinoma	9.57	4.50	20.21	9.50
12	45	6.5	0.49	*E. coli* + Anth.	450.0	Transitional carcinoma	4.08	2.00	28.57	14.00
13	38	6.0	1.00	*E. coli* + Anth.	750.0	Squamous carcinoma	10.49	10.50	24.99	25.00
14	49	7.0	0.76	*E. coli* + Pseud.	850.0	Squamous carcinoma	13.15	10.00	57.01	43.00
15	46	6.5	0.84	*E. coli* + Staph.	700.0	Squamous carcinoma	9.84	8.12	30.30	15.00
16	50	7.2	0.27	*E. coli* + Diph.	2,000.0	Adenocarcinoma	40.16	10.83	58.70	15.83
17	48	5.0	0.30	*E. Coli* + Strept	3,000.0	Squamous carcinoma	29.80	9.16	65.00	19.90
18	43	7.5	0.32	*E. coli* + Pseud.	700,000.0	Squamous carcinoma	171.80	55.00	625.00	200.00
19	55	6.8	0.69	Proteus sp.	16.5	Squamous carcinoma	18.45	12.49	2.46	1.66
20	40	6.0	1.30	Klebsiella sp.	95.0	Squamous carcinoma	2.88	3.74	0.76	9.99
21	50	6.7	0.46	Pseudomonas sp.	150.0	Squamous carcinoma	3.26	1.50	9.34	4.30
22	52	7.0	0.69	Klebsiella sp.	150.0	Anaplastic carcinoma	4.42	2.99	4.30	2.91
23	55	5.5	0.23	Pseudomonas sp.	250.0	Adeno carcinoma	81.45	18.75	40.72	9.37
24	51	5.5	1.20	Proteus sp.	320.0	Transitional carcinoma	1.56	1.90	0.41	0.70
25	51	5.4	0.84	Pseudomonas sp.	750.0	Transitional carcinoma	5.45	4.50	11.51	9.50
26	52	6.5	0.8	Klebsiella sp.	250,000.0	Reactive hyperplasia	18.75	15.00	2.75	2.20

Anthracoids = Anth., Pseudomonas = Pseud., Diphtheroids = Diph.

zyme assay [5, 7, 9, 39]. However we demonstrated that intact bacteria do not utilize that particular substrate denoting that their results excluded bacterial contribution to the urinary β-glucuronidase.

The present investigation demonstrated that bacteria showed a lag period of several hours before enzyme activity became detectable. Since 6–8 hr are approximately the maximum time for an individual, normally, to retain urine in the bladder, we attract the attention to the importance of frequent evacuation of the bladders to decrease the possible hazard of liberating carcinogens by the action of bacterial β-glucuronidase.

Table 8. *Incidence of different types of urinary bacteria in bilharzial infested and bladder cancer patients*

Bacterial type	Percentage of incidence	
	Bilharzial (22)	Bladder cancer (26)
E. coli	50.00	69.20
Staphylococci	18.00	11.50
Diphtheroids	18.18	3.80
Pseudomonas sp.	13.60	19.20
Streptococci	13.60	7.60
Klebsiella sp.	9.00	11.50
Salmonella sp.	9.00	—
Anthracoids	—	7.60
Proteus sp.	4.50	7.69

( ) Number of cases.

On the basis of the results represented here the following could be concluded:

Firstly: urinary β-glucuronidase is derived from the tissue affected mostly with bilharzial infection such as liver, kidney, bladder mucosa and leucocytes associated with the inflammatory process. Another main source is the bacteria that are usually associated with the bilharzial infestation and are always overlooked in treatment.

Secondly: the relation between the severity and the type of bacterial infection and the level of urinary β-glucuronidase is clearly demonstrated.

Thirdly: the possibility to free conjugated carcinogens, such as *o*-aminophenols derived from tryptophan metabolism [40–42], possibly present in the urine, by the action of β-glucuronidase will be enhanced by storage of bacterial infected urine in the bladder of bilharzial infested patients.

Thus elevation of urinary β-glucuronidase due to organ damage or bacterial infection associated with bilharzial infestation together with other cofactors such as vitamin A deficiency (unpublished data) has to be considered as a factor in the aetiology of bilharzial bladder cancer.

Table 9. *Effect of urine storage on β-glucuronidase activity and bacterial count in urine*

No. of cases	Fresh urine			Stored urine*		
	Bacterial count/ml × 10^3	Activity		Bacterial count/ml × 10^3	Activity	
		pH 5	pH 7		pH 5	pH 7
1	4.5	4.28	3.39	120.0	10.70	142.80
2	1.5	1.88	0.43	44.0	2.62	13.04

Activity expressed as μg *p*-nitrophenol/hr/mg creatinine.
*Urine was stored for 24 hr at room temperature.

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