STUDIES ON THE URINARY EXCRETION OF CERTAIN TRYPTOPHAN METABOLITES BEFORE AND AFTER TRYPTOPHAN LOADING DOSE IN BILHARZIASIS, BILHARZIAL BLADDER CANCER AND CERTAIN OTHER TYPES OF MALIGNANCIES IN EGYPT

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THE significance of certain tryptophan metabolites and their possible role in the production of bladder cancer was first suggested by Dunning, Curtis and Maun (1950), while studying the carcinogenic effect of 2-acetylaminofluorine on rat bladder. Bladder implantation experiments conducted by Allen, Boyland, Dukes, Horning and Watson (1957) have proved the carcinogenic nature of these tryptophan metabolites. Such metabolites were also reported to be present in abnormally high concentration in patients suffering from various malignancies particularly the bladder cancers (Dalglish, 1955; Boyland and Watson, 1956; Price, 1958). The abnormal excretion was made clearer when a single dose of L-tryptophan was given to such patients.

The present work is essentially an extension of the preliminary study previously reported in this Journal (Abul-Fadl and Khalafallah, 1961) concerning the urinary excretion of such tryptophan metabolites by bilharzial and bladder cancer patients in Egypt. The tryptophan loading test has been found useful, and the study was extended to include other cases of malignancy occurring in this country. It would be interesting to find out by such an investigation any possible climatic, nutritional or racial variations in the excretion of these metabolites that might help to explain the abnormally high incidence of certain malignant diseases among the Egyptians, particularly bladder cancer. The latter disease is not so common in other countries which are known to be similarly affected by bilharziasis.

The tryptophan metabolites (anthranilic acid, 3-hydroxy-anthranilic acid, kynurenine, kynurenic acid, xanthurenic acid, indole-3-acetic acid and 5-hydroxy-indole-acetic acid); were determined in urines collected before and after the oral administration of a single dose of 100 mg. of L-tryptophan/kg. body weight.

The results obtained showed characteristic patterns of excretion of these tryptophan metabolites in urines collected from bilharzial patients, bilharzial bladder cancers, and patients suffering from twelve other different types of malignant disease of relatively common occurrence in Egypt.

The tryptophan loading test has proved its value in assessing such urinary excretion patterns.

EXPERIMENTAL

Choice of cases

All cases included in this investigation were carefully selected and examined in the medical, surgical, urology and X-ray departments of the Cairo University

Hospitals (Kasr El-Aini). The diagnosis was reached after careful clinical, X-ray and laboratory investigations. The normal subjects (100 cases) were aged from 18 to 55 years and were mostly without known previous history of bilharzial infection. Urines and stools were examined and proved free from any other parasitic infections.

The bilharzial cases comprised simple and complicated infections. The simple (100 cases), represent the first attack of urinary schistosomiasis. The complicated bilharziasis (100 cases) represent victims of repeated attacks of schistosomiasis accompanied by hepatosplenomegaly.

The bladder cancer cases (100 cases) had a definite bilharzial history. Cystoscopic examinations and laboratory tests on biopsies revealed the presence of bilharzial granulomatous tissues.

Twelve types of malignancy involving tissues other than the bladder, breast (8 cases), cervix (5 cases), prostate (5 cases), lip (5 cases), larynx (4 cases), thyroid (4 cases), tongue (4 cases), brain (3 cases), bone (5 cases), liver (5 cases), bronchus (5 cases), and rectum (4 cases).

Collection of urine

Complete 24 hour urines collected under toluene and preserved in the cold whenever possible, were subjected to routine examination and chromatographic studies. For the determination of 5-hydroxyindole acetic acid, the urines were collected over a mixture of glacial acetic acid and toluene (25 ml. + 5 ml. respectively). Cold storage was also advisable.

In the tryptophan loading test, the fasting patients were given a single dose of 100 mg. L-tryptophan per kg. body weight suspended in gum acacia syrup. The urine was then collected during the following 24 hour period.

METHODS

Colorimetric Determinations

5-Hydroxyindole acetic acid was determined according to the method described by MacFarlane, Dalglish, Dutton, Lennox, Nyhus and Smith (1956). For indole-3-acetic acid, the method of Weissbach, King, Sjoerasma and Uden Friend (1959) was adopted.

The diazotisable aromatic amines (kynurenine and anthranilic acid) and the orthohydroxyamine (3-hydroxyanthranilic acid) were determined by the technique derived by Tompsett (1959). For the determination of kynurenic acid and xanthurenic acid, the method of Satoh and Price (1958) was used.

Chromatographic Study

The technique described by Dalglish (1955) and modified by Boyland and Williams (1956) was applied as follows:

1/50th of the complete 24 hour urine collection was acidified with acetic acid to about pH 4, then centrifuged. The clear supernatant was transferred in 10 ml. portions to a glass tube column 1 cm. in internal diameter, 15 cm. in length, previously prepared to contain a layer of deactivated charcoal one cm. long suspended in 0.5% acetic acid solution over a plug of glass wool. After passing the whole amount of urine through the charcoal, the column was washed with 50 ml.

distilled water, passing aliquots of 10 ml. at a time. Elution of the adsorbed metabolites was then effected by 50 ml. 5% (w/v) aqueous phenol solution also in 10 ml. aliquots.

The eluate was evaporated to dryness under reduced pressure, 10 ml. of pure methanol were added to the residue, stirred, then evaporated again to dryness. The residue was finally dissolved in one ml. distilled water. Using the butanol-acetic acid water system (4 : 1 : 5) (Partridge 1948), the paper chromatographic study was then conducted on 0.05 ml. of this solution. Two standard mixtures, containing 2 and 10 micrograms respectively of each of the investigated metabolites in a total volume of 0.05 ml. were also run parallel to the tested urines on the same chromatograms. After drying at 40° c., the spots of kynurenine, kynurenic acid, xanthurenic acid and 3-hydroxyanthranilic acid were first detected and traced under ultra-violet light (3655 Å). The chromatograms were then sprayed with Ehrlich's aldehyde reagent to develop the coloured spots for 5-hydroxyindole acetic acid, kynurenine and 3-hydroxyanthranilic acid.

Semiquantitative evaluation of the chromatographic spots

This was conducted by comparing the areas of the chromatographic spots for the metabolites obtained from the urines with those obtained from the known standards. Area measurements were carefully conducted with a planimeter and the relative concentrations were derived from Fisher's equation (Fisher, Parson and Morrison (1948) as follows :

$$A = b. \log c + d$$

Where A = area of the spot ; c = the concentration of the material on the spot ; b and d are constants which are dependent on the length of the run and condition of colour development as well as on the particular characteristics of the pore pattern of the filter paper sheet used. Since the standard and the unknown were run under exactly the same conditions, the constants b and d can be neglected ; and thus if :

A_1 was the area of a spot from a charcoal urine extract,

C_1 was the concentration of the metabolite content of the spot

A_2 was the area of the corresponding spot in the standard mixture and C_2 was the concentration of the metabolite standard content of the spot, then :

$$\frac{A_1}{A_2} = \frac{\log C_1}{\log C_2}$$

$$\therefore \log C_1 = \frac{A_1 \times \log C_2}{A_2}$$

The determination, however, was approximate and several factors were involved including the length of run, irregularity in paper structure and area of the applied substance to the paper.

RESULTS AND DISCUSSION

The results of the colorimetric, fluorimetric and chromatographic study of the investigated metabolites are presented in the tables as follows :

The chromatographic separation of these metabolites gave sharp well identified spots for kynurenine, anthranilic acid, 3-hydroxyanthranilic acid and 5-hydroxy-

TABLE I.—*Colorimetric and Fluorimetric Determinations of the Urinary Excretion of Certain Tryptophan Metabolites in Normal, Bilharzial and other Egyptians with Various Malignant Diseases Before the Oral Administration of L-Tryptophan*

Diagnosis	No. of cases	Tryptophan metabolite excretion (mg./24 hr.)											
		5-Hydroxy indole-acetic acid			Indole-3-acetic acid			Anthranilic acid			3-Hydroxy-anthranilic acid		
		Range	Average		Range	Average		Range	Average		Range	Average	
Normal	100	3.2-13.3	8.6		3.13-16.5	7.0		0.14-0.9	0.5		0.45-2.4	1.4	
Simple bilharziasis	100	12.9-40.7	22.6		3.22-13.4	6.2		0.12-1.02	0.6		1.0-3.2	2.1	
Bilharzial hepatosplenomegaly	100	13.4-36.2	21.7		2.45-9.63	5.4		0.12-0.99	0.5		1.36-3.59	2.2	
Bilharzial bladder cancer	100	19.0-104.2	35.6		2.4-13.9	5.5		1.01-4.89	2.8		1.43-16.2	8.0	
Cancer cervix	5	3.2-8.6	6.4		4.8-10.6	7.5		0.90-1.21	1.1		0.45-1.82	1.2	
Cancer breast	8	3.4-6.8	4.8		6.9-11.4	9.7		0.38-1.22	0.8		2.1-4.92	3.2	
Cancer prostate	5	17.6-23.7	21.0		2.8-4.3	3.5		0-0.18	0.1		0-0.25	0.2	
Cancer lip	5	38.7-82.4	59.6		7.8-10.4	9.0		0.04-0.91	0.6		1.2-2.1	1.6	
Cancer larynx	4	72.0-115	90.0		1.89-3.22	2.6		0.09-0.14	0.1		0.21-0.91	0.4	
Cancer thyroid	4	7.6-10.2	8.9		5.6-8.45	7.2		0.58-1.0	0.8		1.2-2.3	1.9	
Cancer tongue	4	3.1-4.0	3.7		5.2-13.8	9.5		0.67-1.12	0.9		1.85-2.42	2.1	
Malignant tumours of the brain	3	33.2-51.8	41.7		3.9-5.15	4.4		0.95-1.23	1.1		2.2-3.8	2.9	
Cancer bone	5	19.8-27.6	24.7		3.9-6.2	4.8		0.12-0.85	0.3		1.0-1.42	1.1	
Liver cell sarcoma	5	18.5-24.5	21.5		3.9-5.6	4.7		0.45-0.92	0.7		2.2-3.0	2.7	
Cancer bronchus	5	21.5-28.0	23.6		4.0-6.1	4.9		0-0.2	0.04		0.15-0.32	0.2	
Cancer rectum	4	2.8.4.1	3.5		6.9-8.2	6.1		0.18-0.42	0.2		0.38-0.8	0.4	

Diagnosis	No. of cases	Kynurenine			Kynurenic acid			Xanthurenic acid		
		Range	Average		Range	Average		Range	Average	
		Range	Average		Range	Average		Range	Average	
Normal	100	4.0-9.2	5.9		6.5-15.9	11.7		4.0-13.0	9.1	
Simple bilharziasis	100	4.12-9.9	6.4		7.6-19.9	13.6		6.8-16.1	11.6	
Bilharzial hepatosplenomegaly	100	5.2-13.0	6.9		13.0-24.2	16.8		10.4-18.9	14.4	
Bilharzial bladder cancer	100	4.4-14.6	9.5		3.2-10.8	5.7		1.5-8.5	4.8	
Cancer cervix	5	6.8-9.4	8.2		8.5-13.2	10.5		7.8-10.4	9.3	
Cancer breast	8	4.4-10.1	7.2		7.6-11.7	9.4		6.4-8.4	7.6	
Cancer prostate	5	6.1-11.2	8.8		10.4-12.1	11.5		4.0-6.2	4.9	
Cancer lip	5	3.2-10.4	6.6		10.7-12.5	11.6		7.6-9.5	8.6	
Cancer larynx	4	3.29-7.4	5.0		8.4-11.2	9.9		6.0-8.92	7.3	
Cancer thyroid	4	4.82-6.15	5.6		10.9-12.2	11.5		9.5-10.2	9.9	
Cancer tongue	4	5.7-8.2	7.2		11.8-13.6	12.4		7.6-10.2	9.4	
Malignant tumours of the brain	3	4.9-7.2	5.9		12.0-13.2	12.4		10.0-11.5	10.5	
Cancer bone	5	6.9-8.2	7.3		11.2-13.2	12.2		8.0-10.2	9.4	
Liver cell sarcoma	5	4.2-6.2	5.0		6.8-10.1	8.3		4.1-6.0	5.0	
Cancer bronchus	5	8.2-11.2	10.0		4.7-12.4	9.8		8.5-11.9	10.5	
Cancer rectum	4	2.9-6.11	3.9		4.7-11.2	6.6		4.0-8.1	4.5	

TABLE III.—*The Relative Rates of Excretion of the Tryptophan Metabolites Before and After L-Tryptophan Loading Test (A/B Ratio—After/Before Test) in Normal Bilharzial and Different Malignancies*

The average A/B ratios are calculated from actual figures obtained in each experiment as illustrated in Table II

Diagnosis	5-Hydroxyindole acetic acid		Indole-3-acetic acid		Anthranilic acid		3-Hydroxy-anthranilic acid		Kynurenine		Kynurenic acid		Xanthurenic acid	
	average A/B ratio	A/B	average A/B ratio	A/B	average A/B ratio	A/B	average A/B ratio	A/B	average A/B ratio	A/B	average A/B ratio	A/B	average A/B ratio	A/B
Normals	1.9	.	1.9	.	1.8	.	1.7	.	2.3	.	7.0	.	4.8	.
Simple bilharziasis	1.8	.	1.9	.	1.8	.	2.0	.	2.8	.	8.6	.	5.3	.
Bilharzial hepatosplenomegaly	2.0	.	1.9	.	1.9	.	2.0	.	2.8	.	8.6	.	5.0	.
Bilharzial bladder cancer	1.9	.	1.9	.	2.7	.	5.2	.	3.3	.	9.7	.	8.4	.
Cancer cervix	1.7	.	2.0	.	1.6	.	2.2	.	3.0	.	8.5	.	5.3	.
Cancer breast	1.7	.	2.0	.	1.8	.	2.1	.	2.8	.	9.2	.	6.0	.
Cancer prostate	1.9	.	2.2	.	0.6	.	1.0	.	3.0	.	9.3	.	4.8	.
Cancer lip	2.1	.	1.8	.	1.7	.	2.1	.	3.1	.	8.4	.	4.9	.
Cancer larynx	8.4	.	1.8	.	1.7	.	1.6	.	2.3	.	6.3	.	5.0	.
Cancer thyroid	1.9	.	2.0	.	1.6	.	2.0	.	2.5	.	8.0	.	5.2	.
Cancer tongue	1.8	.	1.8	.	1.6	.	1.8	.	2.7	.	8.1	.	5.7	.
Malignant tumours of the brain	2.1	.	1.9	.	1.6	.	1.9	.	2.9	.	11.1	.	5.2	.
Cancer bone	1.9	.	2.0	.	1.7	.	1.8	.	2.7	.	9.7	.	7.0	.
Cancer liver	1.9	.	2.0	.	1.8	.	1.5	.	2.8	.	8.3	.	5.8	.
Cancer bronchus	1.8	.	2.0	.	0.1	.	1.6	.	2.7	.	9.2	.	5.5	.
Cancer rectum	1.8	.	1.9	.	1.5	.	1.8	.	2.9	.	9.0	.	6.5	.

TABLE IV.—*Showing the Average Urinary Excretion of Tryptophan Metabolites (chromatographic determination) in Normal and in Pathological Cases Before and After the L-Tryptophan Loading Test*

Diagnosis	No. of cases	Tryptophan metabolite excretion (mg./24 hr.)									
		5-Hydroxy-indole acetic acid		3-Hydroxy-anthranilic acid		Kynurenine		Kynurenic acid		Xanthurenic acid	
		Before	After	Before	After	Before	After	Before	After	Before	After
Normals	8	..	8.8	..	2.0	4.6	13.5	5.9	38.6	2.4	10.5
Simple bilharziasis	8	10.8	21.9	1.9	3.6	4.1	10.0	7.6	65.0	2.2	10.9
Bilharzial hepato-splenomegaly	8	12.3	25.0	1.7	3.3	3.4	7.6	8.1	61.1	3.0	12.9
Bilharzial bladder	8	18.2	30.6	4.6	22.1	3.8	11.1	4.3	38.0	1.6	7.5
Cancer											
Cancer cervix	3	..	8.7	..	2.4	4.2	13.9	7.9	56.3	2.4	8.4
Cancer breast	3	..	..	1.5	3.4	4.0	11.6	6.1	62.8	1.8	7.6
Cancer prostate	3	14.7	28.0	..	..	5.5	15.7	7.9	68.0	1.4	6.0
Cancer lip	3	38.7	81.0	..	0.7	5.0	15.1	7.2	60.0	1.9	8.5
Cancer larynx	3	57.7	105.3	..	..	3.6	10.9	5.9	45.0	2.1	8.6
Cancer thyroid	3	..	9.3	..	2.2	3.4	9.9	8.6	54.3	2.4	9.3
Cancer tongue	3	..	..	..	1.5	4.0	14.9	7.5	64.3	2.5	10.7
Malignant tumours of the brain	3	28.9	56.9	1.9	3.6	3.3	11.1	7.6	90.8	2.6	10.3
Cancer bone	3	15.7	30.3	..	..	4.8	13.4	8.0	74.0	2.3	11.1
Cancer liver	3	14.5	28.6	..	2.1	3.3	9.6	6.5	48.3	1.2	6.1
Cancer bronchus	3	15.8	30.3	..	..	6.4	20.2	5.5	48.6	2.3	10.5
Cancer rectum	3	..	..	..	..	2.9	10.2	5.3	42.6	1.5	7.2

indole acetic acid. Kynurenic and xanthurenic acids on the other hand, the R_f values of which are so close to each other (0.55 and 0.56 respectively in butanol-acetic acid water (Partridge, 1948)), were almost overlapping, and thus their proper semiquantitative estimation was not satisfactorily achieved. Nevertheless, an approximate idea of the quantities excreted of these two metabolites in the urine was always reasonably obtained by a more careful chromatographic study. Indole-3-acetic acid, however, was not chromatographically detected in any urine whether normal or pathological, before or after administration of tryptophan. This metabolite seems to have a high affinity for the deactivated charcoal on which it was easily adsorbed but not possibly eluted by the phenol, and its quantitation was thus confined to the colorimetric methods. Besides the latter metabolite, the detection of 5-hydroxyindole acetic acid, anthranilic acid and 3-hydroxyanthranilic acid in the urines collected from normal as well as from certain pathological cases, was not amenable to the above chromatographic technique.

It seems that these metabolites were excreted in such low concentrations as to escape detection in the eluate. Their quantitation was also confined to the colorimetric methods.

The results obtained for each of the above mentioned metabolites are discussed below.

Anthranilic acid

The normal excretion of anthranilic acid as detected colorimetrically was found to range from 0.14 to 0.9 with an average of 0.5 mg./24 hour urine.

In bilharziasis, whether simple or complicated, no apparent variation from normal was detected. In bladder cancer, the average excretion of this metabolite

was found to be increased to about six times the average normal value. No rise to such an extent was observed in any of the malignancies studied which involve organs other than bladder. For example, in cancer of the cervix, the thyroid, the breast and in malignant tumours of the brain, there was mild elevation in the urinary excretion of anthranilic acid but the highest level was only a little above the maximum normal excretion.

After the oral administration of tryptophan the average rate of excretion of anthranilic acid in the urine as compared with the same before loading test, i.e. the A/B^* ratio, was 1.8 in normals, 1.8 to 1.9 in bilharzial infection, while in bladder cancer, this ratio was 2.7, although it did not exceed 1.8 in any of the other forms of cancer examined. This shows clearly that although the anthranilic acid is only excreted in small quantities in urines of normals and malignancies, yet its relative increase is, so far, a characteristic feature of bladder cancer.

Such results for bilharzial bladder cancer seem to be in agreement with those described by Boyland and Watson (1956) and Price (1958), working on bladder cancer in England and America respectively where this disease is apparently of industrial origin. Thus the metabolic turnover in this disease, as shown by the anthranilic acid excretion, seems to be the same whether the suspected cause is industrial or bilharzial. The only difference is that the figures mentioned by the British and American authors are slightly higher than those found in this country. This also applies to the level in normal subjects. Thus the relatively low anthranilic acid urinary excretion in this country might possibly be attributed to differences in climatic, racial or nutritional habits.

3-Hydroxyanthranilic acid

The normal urinary excretion of 3-hydroxyanthranilic acid was found to range from 0.45 to 24 mg. with an average of 1.4 mg./24 hour urine. In bilharziasis, whether simple or complicated, excretion of this metabolite was increased to about twice the normal average.

In bladder cancer, however, the average urinary excretion of this metabolite was found to be increased to about 8 times the average normal values.

In cancers of the breast, malignant tumours of the brain and reticulum cell sarcoma of the liver, the urinary excretion of 3-hydroxyanthranilic acid ranged from 2.7 to 3.2 mg./24 hour and the average excretion level was only twice as much as the normal.

In malignancies involving the prostate, the lip and the larynx the average urinary excretion of this metabolite was greatly decreased below the normal average level, particularly in prostatic cancer where urinary 3-hydroxyanthranilic acid was almost absent in some cases.

Other types of cancer did not seem to produce any significant variation from the normal level of excretion.

Following the oral administration of tryptophan the average rate of excretion of 3-hydroxyanthranilic acid, as compared with same before the loading test (i.e. A/B ratio), was 1.7 in normals, 2 in bilharzial infection, and 5.2 in bladder cancer.

In malignancies involving the cervix, breast, lip and thyroid this ratio ranged

* A/B ratio = After/Before test.

from 2.0 to 2.2, while in the remaining types of malignancies the ratio was within normal limits.

This shows clearly that the maximum excretion of 3-hydroxyanthranilic acid is characteristically obtained in bilharzial bladder cancer, although the amount usually excreted is again less than that recorded abroad, possibly for the same reasons mentioned under anthranilic acid.

These results are in fair agreement with those reported by Boyland (1956) on the aniline bladder cancer and bladder cancer in the general population of England.

Hence, the tryptophan metabolic disorder of this disease, as far as 3-hydroxyanthranilic acid excretion is concerned seems to be the same whether the cause is industrial or bilharzial.

Indole-3-acetic acid

The normal urinary excretion of indole-3-acetic acid as determined colorimetrically ranged from 3.13 to 16.5 mg. with an average of 7.0 mg./24 hour urine. The present chromatographic technique however failed to detect this metabolite either in normals or in any of the pathological urines investigated.

No significant variation in the urinary excretion of this metabolite was noticed in bilharziasis, whether simple or complicated, or in bilharzial bladder cancer.

In cancer of the prostate and of the larynx the average urinary excretion of indole-3-acetic acid was markedly below the normal average. The other types of cancers examined, on the other hand, did not show any significant variation from the normal level of excretion of this metabolite.

After the tryptophan loading test the indole-3-acetic acid excretion varied in normal subjects from 8.5 to 17.95 mg. with an average of 12.5 mg./24 hour (A/B ratio = 1.9).

In bilharziasis, whether simple or complicated, and in bilharzial bladder cancer, the picture remained still within the normal range. On the other hand, patients with malignancies involving the breast, the tongue, the lip, the cervix, the rectum and the thyroid gland seem to excrete more indole-3-acetic acid than did the normal subjects after the oral tryptophan test (A/B ratio ranged from 1.8 to 2.2).

In cases of cancer of the prostate and cancer of the larynx the excretion of indole-3-acetic acid was still below that of the normal subjects similarly treated. The remaining types of cancer showed no variation from normals after tryptophan administration. Thus the tryptophan loading test confirmed the characteristic property of prostate and larynx cancer as regards the diminished excretion of indole-3-acetic acid in urine. It also revealed the tendency of certain other malignancies towards increased excretion of this metabolite in the urine.

5-hydroxyindole acetic acid

The normal urinary excretion of 5-hydroxyindole acetic acid varied from 3.2 to 13.3 mg. with an average of 8.6 mg./24 hour urine. In simple and complicated bilharziasis the average urinary excretion of this metabolite ranged from 21.7 to 22.6 mg./24 hour urine, showing a level about 3 times as high as that of the normal.

In bilharzial bladder cancer on the other hand, the average excretion of this metabolite was increased to about 4 times the normal average.

As for the other malignant diseases, urinary 5-hydroxyindole acetic acid was

considerably increased in larynx cancer, lip cancer and malignant tumours of the brain to an extent exceeding that found in bladder cancer. The average excretion of this metabolite in the above three conditions with respect to the normal average excretion amounted to about 10, 7 and 5 fold increases respectively. The excretion of this metabolite in cancer of bone, liver and bronchus was of about the same order as that in the case of bilharziasis. In cancer of the rectum, tongue and breast, on the other hand, the level of excretion of 5-hydroxyindole-acetic acid was found to be persistently on the lowest side of the normal range.

Boyland, Gasson and Williams (1957) have also reported on the increase in the excretion of this metabolite in cancer of the larynx and bronchus. It is of interest to mention here that, during our studies on pellagra in Egypt, we have noticed a marked increase in the urinary excretion of this metabolite in untreated pellagra cases to an extent comparable to that found in bilharziasis, which diminished after treatment with adequate doses of nicotinamide (work in progress, unpublished yet).

The tryptophan loading test showed also a pronounced increase in the excretion of this metabolite in the urine of the above mentioned patients with an already elevated excretion before the test, but the A/B ratio was only a little above normal except for the cancer of the larynx cases where the ratio was more than 4 times that of the normal.

The increase in the excretion of 5-hydroxyindole-acetic acid has been said to be pathologically related to the tumour origin since the bladders of bilharziasis usually show squamous metaplasia and the cancer is usually a squamous cell carcinoma. The larynx, lip, liver and bronchus all develop squamous cell carcinoma and produce marked increase in the urinary excretion of 5-hydroxyindole acetic acid.

Cancer of the cervix, however, though frequently of squamous cell type, yet did not show any increase, whereas the brain which is ectodermal in origin and with a different histological type of tumour from the squamous cell carcinoma showed marked increase in the urinary excretion of this metabolite.

This shows clearly that the type of the malignant cell does not seem to be a primary factor affecting the urinary increase in the 5-hydroxyindole-acetic acid excretion, and that certain other factors which possibly involve the cell nutrition and metabolism must be considered.

Kynurenine

The normal urinary excretion of kynurenine was found to range from 4 to 9.2 mg. with an average of 5.9 mg./24 hour urine. Somewhat lower results were obtained by the chromatographic technique (range 3.0 to 6; average 4.6 mg./24 hour urine). In simple bilharziasis and bilharzial hepatosplenomegaly no significant deviation from the normal level was detected by either the colorimetric or the chromatographic method. In bladder cancer, an increase in the excretion of this metabolite to about twice the average normal level was found. Slight elevation in the kynurenine excretion was noticed in cancer of the cervix, breast, prostate and bronchus. In cancer of the rectum, however, the excretion level was rather low, whilst in the other types of malignancy examined no significant variation from normal was noticed.

Colorimetric and chromatographic methods of analysis of this metabolite showed agreement, although the values obtained by the latter technique were still lower than those obtained by the former. The values described for kynurenine excretion in industrial bladder cancer (Price, 1958) seem to be near to those recorded in bilharzial bladder cancer in the present work.

After the tryptophan loading test, marked elevation in the urinary excretion of this metabolite was obtained in cancer of the bronchus, cancer of the prostate, cancer of the lip and in bladder cancer in decreasing order though the highest A/B ratio was obtained in the last. In the other types of cancer examined the level of excretion was almost within normal limits.

Kynurenic acid and xanthurenic acid

These two metabolites were determined by fluorimetric and by chromatographic analysis.

Fluorimetrically, the urinary excretion of kynurenic acid in normal Egyptians was found to range from 6.5 to 15.9 mg. with an average of 11.7 mg./24 hour; xanthurenic acid excretion on the other hand ranged from 4 to 13 mg. with an average of 9.1 mg./24 hours.

Using the chromatographic technique, kynurenic acid was detected in the urine of normal subjects in amounts ranging from 5.1 to 6.9 mg. with an average of 5.9 mg./24 hours.

Xanthurenic acid was excreted from 1.6 to 2.8 mg. with an average level of 2.4 mg./24 hours.

The marked discrepancy in the results between the fluorimetric and the chromatographic analysis is due, as previously mentioned, to the close R_f values of these two metabolites, besides the possible loss involved in the latter technique.

In simple bilharzial infection, a slight elevation in the level of both the metabolites was effected. The elevation becomes more evident in bilharzial hepatosplenomegaly, in which the average excretion level reached one and half times the normal average excretion for each of the two metabolites.

In bladder cancer, on the other hand, the average excretion is surprisingly reduced below the normal average level of excretion of both metabolites.

Carcinomas of larynx and breast showed also relatively lower levels of excretion, while other types of malignancy did not show significant variation from the average normal values.

The tryptophan loading test in normal subjects resulted in an excretion of 65 to 85 mg. of kynurenic acid with an average of 74.3 mg./24 hours, while xanthurenic acid excretion ranged from 34 to 50 mg. with an average of 45.6 mg./24 hours as determined fluorimetrically. In bilharzial patients, whether suffering from simple bilharzial infection or complicated with hepatosplenomegaly, there was an increase in the urinary excretion of these two tryptophan metabolites amounting to about 1.5 times the normal.

Cancer of the bladder on the other hand showed a relatively lower excretion level than normal for kynurenic acid only, but not for xanthurenic acid. The excretion of the latter after tryptophan in bladder cancer was within the normal level. Cancer of the larynx produced the lowest level of excretion for both kynurenic and xanthurenic acids. Other types of malignancy behaved within the normal range.

SUMMARY AND CONCLUSION

1. Colorimetric, fluorimetric and chromatographic determinations of anthranilic acid, 3-hydroxyanthranilic acid, indole-3-acetic acid, 5-hydroxyindole acetic acid, kynurenine, kynurenic acid and xanthurenic acid have been carried out in normals, patients with bilharziasis (simple and complicated), bladder cancer, and malignancy involving certain organs other than the bladder.

2. The change which is brought about as a result of bilharzial infection, whether simple or complicated, seems to involve an increase in 5-hydroxyindole acetic acid (about 3 times normal), 3-hydroxyanthranilic acid (about doubled), kynurenic acid and xanthurenic acid (about 1.5 times). No apparent change was detected in the excretion of indole-3-acetic acid, anthranilic acid and kynurenine.

3. In bilharzial bladder cancer the characteristic pattern of excretion of these metabolites can be summarized as follows :

(a) A marked increase in : 3-hydroxyanthranilic acid (about 8 times), anthranilic acid (about 6 times) ; 5-hydroxyindole acetic acid (about 4 times) ; and kynurenine (about 2 times).

(b) Kynurenic and xanthurenic acids showed characteristic diminution below the normal average (about 50% reduction).

(c) Indole-3-acetic acid excretion showed no significant variation from the average normal.

4. The study of the levels of excretion of these metabolites in malignant disease involving bone, the brain, breast, bronchus, cervix, larynx, lip, liver, prostate, rectum, thyroid gland and tongue showed a marked elevation in the level of excretion of 5-hydroxyindole acetic acid in relation to the average normal excretion per 24 hours (larynx cancer (10 times), lip cancer (8 times) and malignant tumours of the brain (5 times)).

5. The characteristic rise in the urinary excretion of anthranilic acid and 3-hydroxyanthranilic acid, together with the diminution in the excretion of kynurenic acid and xanthurenic acid which was reported in bladder cancer, was not detected in any of the other forms of malignancy which were examined.

6. The tryptophan loading test was conducted on a number of cases representing each group of the above mentioned normal and pathological cases. The test has proved its value in assessing the above mentioned findings. This was particularly evident in bilharziasis, cancer of the bladder and cancer of the larynx.

7. The excretion pattern of the above mentioned tryptophan metabolites in bilharzial bladder cancer in Egypt seems, so far, to be close to that described by other workers for industrial bladder cancer abroad.

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