

IMMUNODIAGNOSIS OF PRECANCEROUS CHANGES  
IN THE MUCOUS MEMBRANE OF THE URINARY  
BLADDER BY DETERMINATION OF THE SERUM  
3-HYDROXYANTHRANILIC ACID

T. A. Korosteleva, B. V. Klyucharev,  
A. T. Belokhvostova, A. A. Syromyatnikov,  
and T. A. Benteleva

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Examination of workers in contact with dyes containing radicals of carcinogenic compounds revealed disturbances of the mucous membrane of the urinary bladder (papillary changes, chronic cystitis, trigonitis). Immunoserologic investigation of the blood serum of these workers by means of an immunologic method suggested for the diagnosis of carcinoma of the urinary bladder revealed 3-hydroxyanthranilic acid, a tryptophan metabolite, in the blood of some of the workers as a hapten. The frequency of discovery of this metabolite, like the frequency of detection of pathological changes in the mucous membrane of the urinary bladder, depended on the degree of contact of the workers with the dyes. It is concluded that the immunologic determination of 3-hydroxyanthranilic acid can be used for the detection of persons with a risk of developing occupational bladder tumors.

KEY WORDS: urinary bladder; precancerous state; 3-hydroxyanthranilic acid; antibodies; diagnosis.

For the timely treatment and prevention of precancer and cancer of the urinary bladder in workers in contact with aniline dyes containing radicals of carcinogens, a method enabling the detection of early forms of these diseases is essential. The writers have proposed and conducted clinical trials of a new immunologic method for the diagnosis of carcinoma of the urinary bladder [5, 6]. The method consists in principle of recording a protein antigen in the patients' blood containing 3-hydroxyanthranilic acid (3HAA), a carcinogenic tryptophan metabolite, as hapten. The development of this method was based on data in the literature showing pathologically increased excretion of 3HAA in the urine of patients with carcinoma of the bladder [1, 7] and the results of experimental studies of the immunology of the early stages of experimental chemical carcinogenesis conducted in the writers' laboratory [2]. The endogenous carcinogen 3HAA, as well as exogenous carcinogens studied previously, such as dimethylaminoazobenzene, benzidine, o-aminoazotoluene, and 2-naphthylamine, are active haptens in the carcinogen-protein antigenic complex, and they can be found in the body with the aid of specific immune sera.

Having considered these facts and shown that 3HAA can be detected as a circulating antigen in the serum of experimental animals in the early stages of carcinogenesis [4], the writers investigated the use of this test for the diagnosis of occupational precancerous and cancerous diseases of the urinary bladder.

#### EXPERIMENTAL

The investigation was carried out on 110 workers, aged from 32 to 65 years, in four departments (dyeing, printing, and folding departments and the dye preparing department) of a textile factory. Depending on

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TABLE 1. Immunologic Determination of 3HAA in Blood Serum and Results of Urologic Examination of Textile Workers

Group	Changes in bladder mucosa				Total
	chronic cystitis	papillary growths	trigonitis	normal	
1	14/23	1/1	4/4	1/4	20/32
2	10/17	3/3	3/11	3/10	19/41
3	5/19	—	3/7	0/6	8/32
4	1/3	—	—	0/2	1/5
Total	30/62	4/4	10/22	4/22	48/110

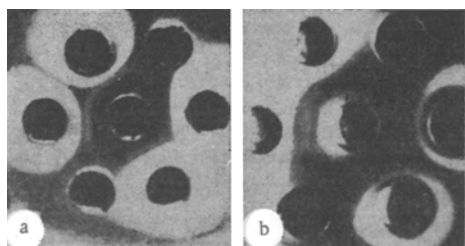


Fig. 1. Determination of 3HAA in blood serum of textile workers: a) worker having direct contact with dyes (group 1) and with papillary changes in the bladder mucosa; b) workers having indirect contact with dyes (group 2); 1) rabbit serum containing antibodies against 3HAA; 2) blood serum of workers; 3) blood serum of healthy donor.

Comparative analysis of the results of the immunologic tests on the blood serum of the workers and the results of their urologic examination definite correlation was found between the results obtained by the two methods. If the changes in the bladder mucosa were of the same pattern, 3HAA was detected more often in workers with a higher degree of contact with dyes containing radicals of carcinogens.

Preliminary observations showed that 3HAA could not be found in the serum of patients with chronic cystitis with no history of contact with dyes. Its appearance in the serum of workers with no cystoscopic evidence of changes in the mucosa can be explained as follows. Disturbances of tryptophan metabolism revealed by the highly sensitive immunologic method, that later may go on to tumor development, precede visible changes in the mucous membrane. This suggestion is confirmed by the fact that 3HAA was found in the blood serum of the workers of groups 1 and 2, with the closest contact with dyes, despite a healthy state of the bladder mucosa, and it was not found in the serum of the workers of groups 3 and 4, with less contact with dyes.

It can be concluded from these findings that although no clinically clearly manifested occupational bladder carcinoma was found among the groups of workers studied it can be postulated on indirect grounds that the determination of 3HAA by an immunologic method in the serum can be used as a test for the detection of early precancerous occupational changes in the bladder mucosa. These indirect grounds are as follows: 1) the absence of 3HAA in the serum of healthy donors; 2) the detection of 3HAA in the serum of patients with carcinoma of the bladder; 3) correlation between the frequency of appearance of 3HAA in the blood of the workers and the degree of their contact with dyes containing radicals of carcinogens; 4) correlation between the frequency of detection of 3HAA in the workers' blood and the degree of the changes in their bladder mucosa; 5) correlation between the frequency of appearance of 3HAA in the blood and changes

their degree of contact with dyes the subjects were divided conventionally into four groups: 1) direct contact with dyes; 2) indirect contact (work with dyed material during its fabrication); 3) indirect contact (work with the finished, dyed material); 4) no contact. All the women received a urologic examination (cystoscopy). For immunologic tests on each worker blood was taken from a vein. 3HAA was determined in the blood serum by the counterdiffusion method in agar using immune rabbit sera containing antibodies against 3HAA [5]. Sera of healthy donors from the First Leningrad Blood Transfusion Station were used as the control. The results were subjected to statistical analysis by the alternative variance method.

## RESULTS

As Table 1 shows, the results of the urologic examination of the women showed a tendency toward an increase in the number of persons with changes in the bladder mucosa with an increase in the degree of contact with dyes. The relationship between the frequency of pathological changes in the bladder mucosa and the degree of contact of the workers with dyes is statistically significant ( $P < 0.001$ ).

3HAA was found in the blood serum of 48 of 110 subjects examined. Its presence was shown by the formation of a precipitation line by interaction between the blood sera of the workers and immune rabbit sera containing antibodies against 3HAA hapten, accompanied by the absence of such a line when the same immune sera was tested with the blood serum of healthy donors (Fig. 1). The higher the degree of contact with dyes, the more frequently was 3HAA found in the workers' blood serum ( $P < 0.01$ ).

discovered in the bladder mucosa, on the one hand, and the degree of contact with dyes on the other hand; 6) the detection of 3HAA in the blood of workers in direct contact with dyes and having a normal bladder mucosa.

The use of the suggested immunologic test may be of great practical importance as a means of detecting persons with an increased risk of development of occupational bladder tumors.

#### LITERATURE CITED

1. A. T. Zasyпка, Vopr. Onkol., No. 7, 108 (1969).
2. T. A. Korosteleva, Changes in Tissue Antigens during Experimental Carcinogenesis [in Russian], Leningrad (1966).
3. T. A. Korosteleva and A. T. Zasyпка, Vopr. Onkol., No. 9, 53 (1968).
4. T. A. Korosteleva and A. T. Zasyпка, Vopr. Onkol., No. 10, 47 (1970).
5. T. A. Korosteleva, B.V. Klyucharev, and A. T. Belokhvostova, Vopr. Onkol., No. 3, 59 (1971).
6. T. A. Korosteleva, E. N. Krutova, and A. T. Zasyпка, Vopr. Onkol., No. 2, 44 (1966).
7. J. M. Price, Canadian Cancer Conference (Proceedings), Vol. 6, New York (1966), p. 224.