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ATTEMPTS TO APPLY THE BIOCHEMICAL INDICATORS TO DEFINE ANILINE, NITROBENZENE, AND BENZENE ABSORPTION AMONG THE DYE INDUSTRY WORKERS [POLISH]

Hotrowski J.

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The evaluation of the actual exposure of the workers to intoxi- 299/
cation can rarely be conducted solely by a quantitative examination of the contamination of the air by the toxic compounds. The actual exposure of the worker depends not only upon the concentration of the toxic materials in the air, but also upon the time which the worker spends in the work room. In the evaluation of the exposure in the dye industry there are additional problems connected with the simultaneous appearance of many toxic compounds in the air and with the very nature of the work of the employees who are not only employed with one process or with one production hall. During these processes significant jumps, which occur quite frequently, in the concentrations of the toxic compounds complicate the evaluation of the exposure based on an atmospheric analysis. In addition nitro-compounds and amino-compounds, which represent the most serious risk of intoxication in the dye industry, may also be absorbed through undamaged skin. In general this type of exposure cannot be determined by atmospheric analyses.

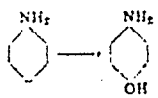
For the above given reasons the evaluation of the actual exposure of the workers should also include a determination of the total quantity of absorbed substance without regard to the absorption pathway. The biochemical tests toward this end must be based on a knowledge of the metabolism of the toxic compound in the body.

At present the simplest compounds most widely employed in the dye industry, aniline and nitrobenzene, are understood the best. In view of the similar nature of the metabolism of these compounds in the

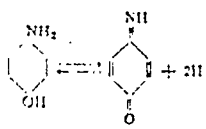
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we shall be limited to a short discussion of the views on the metabolism of aniline.

As may be seen, aniline is oxidized in the body to p-aminophenol:



p-aminophenol, like hydroquinone, is easily oxidized to form quinone imine:



This reaction is reversible. The formation of methemoglobin in the blood is attributed to the oxidative action of quinone imine.

Until recently the following view predominated: one of the metabolites, released in considerable quantities, is p-aminophenol (in man and herbivorous animals). Williams generalizes in his 1947 monograph concerning research in this area, that p-aminophenol is released in the urine not only in the free form but also as acetylated ethyl ether sulfate and probably glucuronide. The other research of Smith and Williams on the metabolism of aniline in rabbits has shaken up the established views. These same authors confirmed the following aniline metabolites in the urine of rabbits: aniline (6.5%), o-aminophenyl glucuronide--frequently acetylated (10-15%), sulfated o- and p-aminophenyl and aminoresorcin (25%). In all only approximately 50% of the metabolites are released in the form of identified metabo-

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lites, among which compounds of p-aminophenol predominate. The remaining 50% are probably released in the form of glucuronides, the structures of which are not yet understood. Smith and Williams consider this unidentified compound to be the primary metabolite of aniline.

As may be seen, even in the relatively well studied field of the metabolism of aniline there are still large gaps which require explanation. In regard to some of the nitro-compounds, important in the dye industry (e.g. nitrochlorobenzene), data concerning their metabolism are in general very insufficient.

However, it appears that at the present time we may attempt to utilize some of the better known facts from the field of the metabolism of aniline and nitrobenzene in the body in order to work out absorption indicators. The following phenomena may be included in the tests for processing of these indicators:

1. Methemoglobin appears in the blood during the process of the metabolism of aniline and nitrobenzene in the body;

2. Detoxification products are eliminated in the urine in the form of ethyl ether sulfates and compounds of glucuronic acid;

3. A major portion of the absorbed compound is eliminated in the form of compounds related to p-aminophenol.

Each of these possibilities will be examined in succession.

THE VALUE OF THE DETERMINATION OF METHEMOGLOBIN IN THE BLOOD AS AN ABSORPTION INDICATOR

The examination of the blood for the content of methemoglobin

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THE VALUE OF THE DETERMINATION OF METHEMOGLOBIN IN THE BLOOD AS AN ABSORPTION INDICATOR

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is well known and has been employed for quite some time not only as an indicator for the absorption of nitrobenzene and amino-compounds but also for their action, i.e., as one of the clinical manifestations. In 1949 M. Chwatowa examined the blood of the dye factory employees, whom we have discussed, for the content of methemoglobin. At that time the level of methemoglobin in the blood of a majority of the workers employed in the production of nitro-compounds and amino-compounds was determined to be from 3-12%.

Unfortunately, this test was not designed to detect the quantity of absorbed methemoglobin-forming compounds. In view of the large variety of methemoglobin-forming compounds encountered for example among the nitro-compounds, it does not appear possible in general, that beside nitro-compounds, nitrogen oxides which appear in substantial concentrations, could play a role here.

During the course of the present work, preliminary tests were conducted to determine the relationship between the concentration of p-aminophenol in the urine and the content of methemoglobin in the blood 301/ of the workers exposed to aniline and nitrobenzene absorption. The determination of methemoglobin was conducted according to the method of Evelyn and Malloy employing Beckman's spectrophotometry. The determination of p-aminophenol was conducted by the method described in the further course of this paper, assuming the content of p-aminophenol in the urine to be an approximate absorption indicator for both of these materials. The test results are given in Table 1.

As follows from Table 1, the detected quantities of methemoglobin

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Table 1. The concentration of p-aminophenol in the urine and the content of methemoglobin in the blood of 7 employees exposed to the effects of aniline and nitrobenzene.

No.	Conc. of p-aminophenol in the urine (mg/liter)	Content of methemoglobin in the blood (g%)
1	15	0,0
2	20	0,2
3	30	0,1
4	35	0,1
5	95	0,6
6	150	0,7
7	180	0,8

in the blood (above 0.2 g%) appear only in conjunction with massive exposure. Therefore, methemoglobin may not be employed as an absorption indicator for nitro-compounds and amino-compounds at low concentrations. This is established by industrial standards. This determination does not reduce the significance of this test in clinical examinations for conditions of nitro-compound and amino-compound intoxication.

THE APPLICATION OF THE DETERMINATION OF THE ESTERS OF SULFURIC ACID

Quantitative changes, like those which occur during the elimination of related compounds (ester sulfates and compounds of glucuronic acid), have until the present time been little employed. The existing difficulties are due to the fact that the elimination of compounds related to sulfuric acid and glucuronic acid is a phenomenon which occurs under physiological conditions. Therefore, changes in the quantities of the eliminated related compounds may be observed only in conjunction with large concentrations of the toxic compounds.

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In the toxicological examinations for the elimination of the esters of sulfuric acid one of the methods for the determination of exposure to benzene was employed. The increase in ester sulfates observed in regard to benzene exposure is connected with the bonding of the metabolite benzene-phenol. These changes are usually determined by designating the percentual proportion of inorganic sulfates to the total quantity of sulfates in the urine:

$$X = \frac{\text{inorganic sulfates}}{\text{total sulfates}} \times 100$$

Under physiological conditions this ratio is usually 80-95%. At atmospheric benzene concentrations above 0.1 mg/liter, a pronounced reduction in the ratio was noted. Based on the above statements it may be stated that these changes would not appear in a method suitable for the determination at nitro-compound and amino-compound concentrations on the order of 0.005 mg/liter. However, because beside exposure to nitro-compounds and amino-compounds in the discussed plant there is also exposure to benzene, especially in the aniline department, we considered the use of this indicator to be desirable *). 302/

Folin's gravimetric method was employed in the tests for sulfates in the urine. The test is based on the conduction of two parallel sulfate determinations: 1) by precipitation with barium chloride in a cold slightly acid medium; on the inorganic sulfates reaction; 2) by precipitating all of the sulfates, inorganic and organic, after

*) The determination of the sulfates in the urine was conducted by assistants of the Institute of Occupational Medicine: Dr. A. Glowacki, J. Posmyklewicz and D. Ader.

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hydrolysis by boiling with concentrated hydrochloric acid.

It was determined in 44 tests on the urine samples of subjects, who were not exposed to benzene absorption (primarily employees of the Institute), that the physiological ratio of sulfates is 78.7 to 96.8%, average: 87.1% (according to Elkins an average of 86%).

In the examinations, which were conducted among the workers of one of our pharmaceutical factories who were exposed to the absorption of benzene vapors in concentrations of 0.15 to 0.4 mg/liter and of 2.8 mg/liter in particularly short lasting processes, the sulfate ratio was from 48 to 77.9%.

In the mentioned dye factory the determination of the sulfate ratio was conducted among the workers of three production departments: nitro-compounds, aniline and anthraquinone. The nature and the intensity of the exposure in these departments were described in the work, which discusses the results of the field experiments. The results of the determinations in these departments are given in Table 2.

Table 2. The sulfate ratio in the urine of the workers of the three production departments of the dye factory.

Department	No. of tests	Sulfate ratio			No. of tests with sulfate ratio below 75%
		From	To	Mean	
Nitro-compounds	6	81.9	82.5	81.4	0
Aniline	6	69.1	84.1	75.0	4
Anthraquinone	4	75.7	86.3	81.6	0
Unexposed subjects	44	78.7	96.8	87.1	0

As may seen from Table 2, a substantial reduction in the sulfate ratio was determined only among the workers of the aniline department;

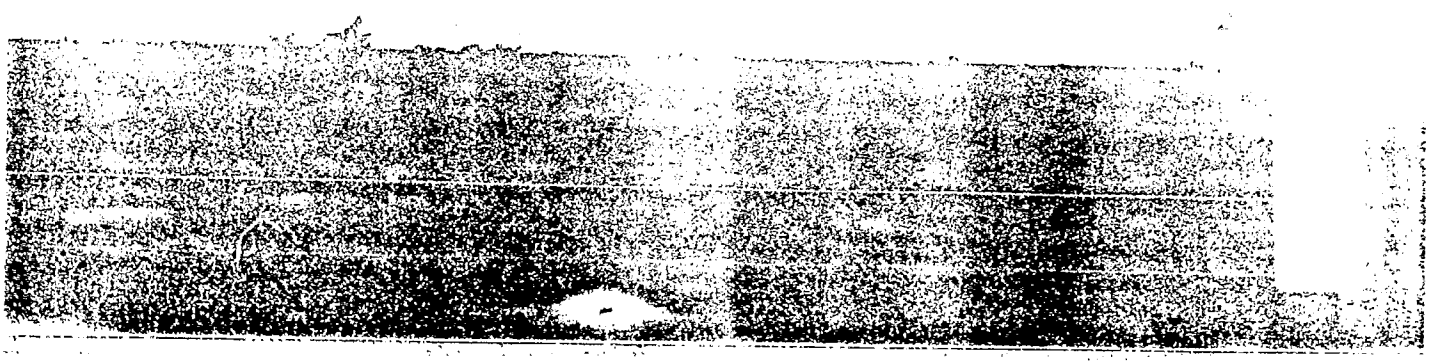
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they were also exposed to benzene in concentrations on the order of 0.2 mg/liter in addition to nitrobenzene and aniline. The tests in this department greatly supplemented the exposure picture determined on the basis of atmospheric analyses. The resultant conclusion, which could not be based on the same atmospheric analyses, may be summarized in the following manner: although the workers of the aniline department do not always remain in the primary production hall, their exposure to the absorption of benzene vapors corresponds to the continuous exposure of the range of the industrial standard or higher. 303/

In the other two departments, where exposure to nitro-compounds exists, no significant reduction in the sulfate ratio was determined in any of the tests (below 75%); however, the mean values are undoubtedly lower than the values determined among the unexposed subjects. In confirmation of the conclusion that the changes in the sulfate ratio could not be employed as an absorption indicator for nitro-compounds and amino-compounds, the values of the sulfate ratio together with the concentration of p-aminophenol in the urine of 4 employees of the anthraquinone department are given in Table 3. These workers were exposed primarily to nitrobenzene; however, they were not exposed to benzene and the other toxic compounds, which are eliminated from the body in the form of sulfates.

THE DETERMINATION OF p-AMINOPHENOL IN THE URINE

In the tests for the determination of exposure to nitrobenzene and aniline the great prospects are attributed to the determination



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Table 3. The concentration of p-aminophenol in the urine and the sulfate ratio of 4 employees of the anthraquinone department.

No.	Conc. of p-aminophenol in urine (mg/liter)	Sulfate ratio (%)
1	60	81,6
2	70	75,6
3	75	86,3
4	400	83,0

of the total quantity of p-aminophenol in the urine. The marked participation of this compound in the metabolism of aniline and nitrobenzene is not disputed; and in addition this compound does not appear in the urine of the exposed subjects.

We have not found any data in the literature concerning the application of this indicator for the quantitative determination of the exposure of subjects under industrial conditions. Of the methods employed in the experimental works on animals the method suggested by D. Robinson, et al. deserves attention. The colorimetric method suggested by these authors for the determination of the total quantity of p-aminophenol in the urine is based on an indophenol reaction, which is conducted after hydrolysis of the urine and after a preliminary substantial dilution. This method, however, permits the determination of the concentration of p-aminophenol in the urine only at concentrations which exceed 100 mg/liter. However, it follows from the approximate calculations that concentrations of p-aminophenol in the urine on the order of 10 mg/liter may be excepted during exposure to the effects of aniline or nitrobenzene in concentrations on the order of the industrial standard (0.005 mg/liter). The method employed in the

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field toxicological tests would, therefore, have to characterize the detectability of the lowest of these orders.

With the determination of p-aminophenol in pure water solutions a detectability of 1 mg/liter or even lower may be achieved without any difficulty. However, the dark color of the urine, subjected to hydrolysis, impedes the determination, during which an inhibition of this reaction also occurs regardless of the masking of the color--which is also probably connected with the effects of the urinary pigment.

I was unsuccessful in working out an accurate method for the determination of low concentrations of p-aminophenol in the urine. In this work we were limited for the meantime to the below described simple, semi-quantitative method, which in my opinion could in general be useful in the evalutationof the workers' exposure. In view of the lack of suitable preparations of p-aminophenol sulfate and glucuronide we determined the hydrolytic conditions on the basis of the work by H.G. Bray, et al. The increase in the detectability of the method, in comparison with the method suggested by Robinson, et al., is achieved by 1) a smaller overall dilution of the urine; 2) filtration of the urine after hydrolysis; 3) taking measurements on an electro-absorptiometer in the little accurate range of low absorption readings.

SEMI-QUANTITATIVE METHOD FOR THE DETERMIANTION OF p-AMINOPHENOL IN THE URINE

The urine was collected from the workers, who were exposed to aniline or nitrobenzene, no sooner than 6 hours after the beginning

Good description of p-aminophenol

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of the workshift on a given day--best immediately after work.

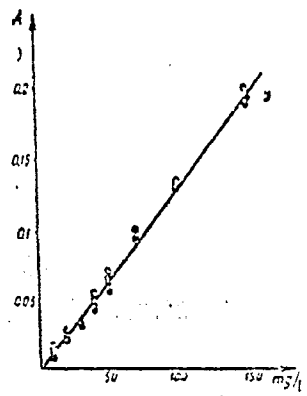


Fig. 1. The relationship of absorption to the concentration of p-aminophenol added to the urine of the 7 unexposed subjects.

Five ml of concentrated hydrochloric acid are added to 10 ml of urine in a test tube; afterward the mixture is heated for 1.5 hours in a boiling water bath. The solution is then poured through a funnel with the usual, qualitative filters into a 100 ml flask; the filter is rinsed with a small amount of water, after which the content of the flask is filled to mark and mixed. Two ml of the solution are pipetted from the flask into two test tubes, of which one serves as the reference for the examination on the electroabsorptiometer; the specific test is conducted on the other test tube. Five ml of water, 1 ml of a 5% water solution of phenol and 2 ml of concentrated ammonia are added to one of the test tubes, 8 ml of water to the other test tube. After stirring the test samples are let to stand for a half hour, after which a blue coloration in the test solutions is indicated on the electroabsorptiometer with the use of a red filter, applied in

Note: No reduction of p-NO_2-Ø-OH

parallel with the prepared test as the reference.

The samples were prepared in an analogous manner; the urine of unexposed subjects was hydrolyzed with hydrochloric acid, and quantities of p-aminophenol were added corresponding to the following concentrations in the urine:

0, 10, 20, 30, 50, 70, 100 and 150 mg/liter.

Thus, the use of p-aminophenol sample solutions in approximately 1 N HCl with a content of 1 mg/ml is advisable. The sample solution should be prepared freshly each time.

The results obtained for the sample tests with the urine of 7 different unexposed subjects are given in Fig. 1. The measurements taken in the urine of each of the subjects are indicated on the graph by a different point.

In the event of the lack of an electroabsorptiometer, the determinations may be made indirectly by a comparison with the range of the samples.

THE CONCENTRATION OF p-AMINOPHENOL IN THE URINE OF THE WORKERS EXPOSED TO ANILINE AND NITROBENZENE 305/

The examinations were conducted among the employees of the nitro-compounds, aniline and anthraquinone departments. The exposure existing in these departments, which was determined on the basis of atmospheric analyses, was described in the work which discusses the results of the field examinations.

a. The nitro-compounds department. The determinations were conducted on the urine of 10 employees of this department in two series.

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The concentration of p-aminophenol was lower than 10 mg/liter in all of the tests.

b. The aniline department. Nineteen determinations were conducted on the urine of 12 employees in 4 series. The results are given in Table 4.

Table 4. Concentrations of p-aminophenol in the urine of the employees of the aniline department.

Conc. of p-aminophenol (mg/liter)	0–10	11–30	31–50	51–70	71–100	101–150	Above 150
No. of tests	1	7	4	3	2	1	1

c. The anthraquinone department. Four determinations were performed. A p-aminophenol concentration of 60-75 mg/liter was determined in three tests, with a concentration of approximately 400 mg/liter in the fourth test.

At the present time it is not possible to determine the overall quantity of aniline or nitrobenzene absorbed during the course of a day's work on the basis of the examination of p-aminophenol in the urine. It could be achieved on the one hand by experimental research, and on the other hand by correlating the field examinations. However, the following hypothesis concerning the interpretation of the obtained results may be conjectured on the basis of the approximate calculations.

Generally speaking, the determined concentrations of p-aminophenol in the urine corroborate the exposure picture based on the analysis of the atmospheric contamination. p-Aminophenol in the urine was not determined in the nitro-compounds department, where the con-

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centrations of nitrobenzene are low. In this case the determinations of p-aminophenol in the urine confirmed the conclusion based on the observation that the high concentrations of nitrobenzene in the nitrocompounds department during the distillation of nitrobenzene are not of great significance, since this process does not require the constant attendance of the operating crew. The constantly quite high concentrations of aniline and nitrobenzene appearing in the aniline department and of nitrobenzene appearing in the anthraquinone department are confirmed in the high concentrations of p-aminophenol in the urine of the employees.

The most frequent concentrations of p-aminophenol in the workers of the aniline department, i.e., within the range of 11-30 mg/liter, but also as high as 70 mg/liter, could be attributed to the absorption of the vapors of these compounds from the atmosphere. However, it does not appear possible that the occasionally encountered higher concentrations of p-aminophenol in the urine could be attributed to the absorption of aniline or nitrobenzene vapors at the concentrations determined in that area. It is highly probable that the absorption pathway in these cases was contaminated skin. The concentration of p-aminophenol in the urine of one employee of the anthraquinone department (400 mg/liter) could be explained only by an unconscious absorption of a large dose of nitrobenzene.

It appears that the frequent examination of the p-aminophenol in the urine of the workers exposed to nitrobenzene and aniline and the conduction of appropriate interviews would help to eliminate some of

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the causes of intoxication.

SUMMARY

A semi-quantitative method of determining the low p-aminophenol concentrations in the urine of workers exposed to aniline and nitrobenzene--has been presented. During the investigations conducted among the workers of three production departments of the dye factory the following conclusions have been reached: 1) p-aminophenol determination in the urine is a valuable supplementation to the image of exposure to nitrobenzene and aniline. The results of determinations are, on the whole, in accord with the concentrations of these compounds in the air. Very high concentrations of p-aminophenol in the urine (above 70 mg/liter) which have been noted in some cases, are considered by the author to be a proof of additional absorption of nitrobenzene and aniline through the skin.

2) Basing on the determination of p-aminophenol in the urine, it is possible to estimate the exposure to nitrobenzene and aniline absorption in the case of concentrations in the air exceeding the industrial norm, but not causing yet the appearance of detectable amounts of methemoglobin in the blood.

3) Changes in the sulfate ratio in urine cannot be an indicator of nitrobenzene and aniline absorption as concerns the concentrations encountered in industry. The determination of the sulfate ratio, on the other hand, may be of great value in estimating the exposure to benzene. In the dye industry, where we are frequently dealing with the mixtures of vapors of various toxic compounds in the air, among

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others with benzene, the use of this test in the case of the known concentrations in the air allows us to estimate adequately the significance of benzene absorption in the general exposure.

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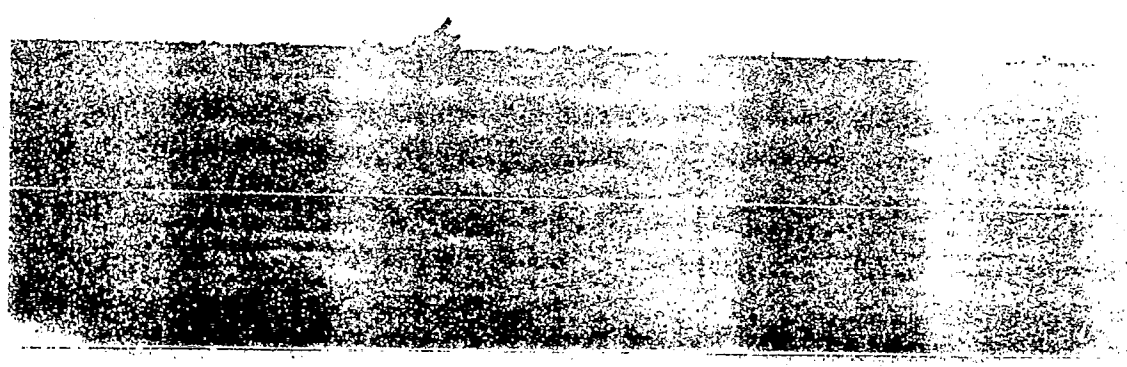
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