



ACTIVATION ANALYSIS OF ARSENIC IN HUMAN HAIR
— SOME OBSERVATIONS ON THE PROBLEM OF
EXTERNAL CONTAMINATION

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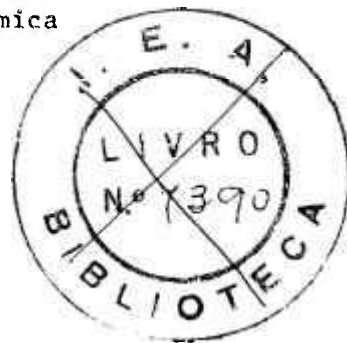
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SUMÁRIO

Arsênico foi analisado, por ativação por nêutrons, nos cabelos de trabalhadores de minas de arsênico, bem como nas unhas, sangue, urina e cabelo do pubis. Várias técnicas de lavagem, incluindo ultra-sons, foram usadas, tentando diferenciar o arsênico no cabelo decorrente do metabolismo do indivíduo daquele decorrente de uma contaminação externa. As mesmas técnicas de lavagem foram também aplicadas a pêlos de coelho ao qual se havia ministrado arsênico através de injeções sub-cutâneas. Conclui-se ser impossível a distinção do arsênico depositado externamente no cabelo, daquele decorrente de eliminação pelo processo metabólico.

RESUMÉ

On a analysé le contenu d'arsenic dans les cheveux des ouvriers de mines d'arsenic, par la méthode d'activation par neutrons. Des résultats sont aussi présentés pour les ongles, le sang total, l'urine et pour les cheveux de la région pubienne.

De différentes techniques de lavage pour les cheveux, le lavage ultra-sonique inclus, on été utilisées afin de distinguer l'arsenic provenant du métabolisme et l'arsenic fixé, extérieurement sur les cheveux.

Les mêmes techniques de lavage on été utilisées pour les poils de lapins qui ont reçu des injections sous cutanées d'arsenite de sodium et il a été vérifié que l'arsenic provenant du métabolisme et celui de contamination externe du cheveux ne peuvent pas être distingués par ces techniques de lavage.

SUMMARY

Hair of arsenic plant workers have been analysed for arsenic by neutron activation methods. Results are also presented for arsenic in nails, whole blood, urine, and pubic hair for those workers. Various washing techniques for hair, including ultra sonics, were applied to the hair, trying to differentiate arsenic from a systemic origin and arsenic externally deposited on the hair.

The same washing techniques were also applied to rabbit's hair to which sodium arsenite sub-cutaneous injections had been applied and it was observed that arsenic from systemic origin and from external contamination, in hair, cannot be differentiated by washing techniques only.

Activation Analysis of Arsenic in Human Hair — Some Observations on the Problem of External Contamination

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INTRODUCTION

Studying the presence of inorganic elements on human hair it is of extreme importance the distinction of elements present as external contamination, from those which have been eliminated, by the hair, through metabolic activity [1].

Arsenic is one of the inorganic elements normally present in human hair, in small amounts. These normal amounts can be modified in cases of arsenic intoxication. It is also possible that arsenic is present in the hair as a consequence of an external contamination and not as consequence of elimination of the element that had been ingested or inhaled. Possibility of this external contamination occurs in circumstances such as those prevailing in arsenic mines or arsenic plants where the hair of workers is in constant contact with arsenic dust. The distinction of these two ways in which arsenic may be present in hairs is, obviously, of high importance to problems of Legal Medicine.

Trying to bring a contribution to the problem of differentiation of the way arsenic may be present in human hair, we decided to use, as working material, hair of people who have been working in arsenic plants for, at least, five years. The amount of arsenic in the hair of those workers is extremely high, around 200 per million. Most of the workers present the classical signs of chronic intoxication and many have a nasal septum perforation.

The mechanism of external contamination might take place in three phases: first, arsenic dust (mostly arsenic trioxide) is deposited on the hair; second, the dust is adsorbed or absorbed by the hair; third, a possible interaction of keratin and arsenic takes place and by which way the arsenic becomes chemically bound to the hair.

Before starting the work with the labourer's hair experiments were carried out concerning contamination, "in vitro", of normal hair, by maintaining the hair in an atmosphere of arsenic trioxide dust for various lengths of time; the same experiment was also carried out with sodium arseniate in solution.

Various washing techniques were then applied to those samples purposely contaminated with arsenic dust or arsenic solution.

The amount of arsenic remaining in the hair, was, at times, very small and the application of classical methods of analysis would be very difficult and lengthy. For this reason it was decided that activation analysis would be the most reliable method to be used.

Activation analysis, as well as other analytical methods, have shown that arsenic is a normal constituent of human body. The following amounts have been reported as being normal, SMALES AND PATE [2]: urine, from 0.13 to 0.33 parts per million (ppm); blood, from 0.09 to 0.50 ppm; hair, from 0.51 to 2.1 ppm; finger nails, from 0.82 to 3.5 ppm; toe nails, from 0.52 to 5.6 ppm.

KINGSLEY ET AL [3] have recorded the normal values for arsenic in various organs: kidney, from 0.026 to 0.037 ppm; liver from 0.030 to 0.039 ppm; heart, from 0.024 to 0.037 ppm; brain, from 0.024 to 0.037 ppm; lungs from 0.018 to 0.029 ppm; leg muscles, from 0.031 to 0.058 ppm.

The presence of arsenic in those organs is a natural consequence of ingestion of vegetables and fruits which have a normal content of arsenic from 0 to 0.7 ppm, possibly with an alteration of the maximum value as a consequence of insecticide sprayings, STOLMAN AND STEWART [4]. Sea food such as oysters, shrimps and others, have an arsenic content from 1.4 to 11 ppm.

In human hair the normal content varies from 0.031 to 0.320 ppm according to YOUNG AND RICE [5]. SMALES AND PATE [2] have found a larger variation which is still considered as normal, i.e., from 0.5 to 2.0 ppm.

Hair has the capability of concentrating arsenic and ALTHAUSEN ET AL [6] have shown that the amount of arsenic present in hair is from four to six times the amount present in the liver, per unit weight, of the same person. This possibility of concentrating arsenic has been considered of utmost importance in cases of poisoning, since the arsenic will still be present in the hair, even when all other traces, exceeding normal amounts, have already been eliminated from the various organs.

Arsenic has a special affinity for all keratinous structures, i.e., nails, skin, and not only for hair, [4]. Arsenic reaches the hair through the blood supply and incorporates irreversibly with the hair and this mechanism becomes a normal way of arsenic excretion from the body.

However, the capability of arsenic retention by keratinous parts is not exclusively dependent on the way the arsenic reaches the tissue, if by the blood supply or by an external contamination. SMITH AND HENDRY [7] have shown that arsenic is adsorbed on hair which has been maintained in contact with a solution of arseniates. STOLMAN AND STEWART [4] have mentioned cases of doubt in exhumation, when arsenic present in the hair could not be definitely ascribed to poison given before death or to contamination from possible soluble compounds of arsenic present in the burying soil. Situations such as this are, obviously, of extreme importance in Forensic Medicine.

In accordance with JERVIS ET AL [8] ingested arsenic is transferred very quickly to nails, skin and hair and the same happens with selenium, mercury and thallium. Beside the determination of these inorganic elements, Jervis and collaborators have also shown, through activation analysis, the presence in hair of copper, zinc, iron, silicon, sodium, vanadium, gold, cobalt, manganese, molybdenum, selenium, chlorine, bromine, iodine, mercury, germanium, chromium and manganese. For this reason, i.e., the excretion function of hair for arsenic as well as for other inorganic elements, hair becomes material of utmost importance for studies of toxicology.

The results reported in the present paper, as well as those previously presented by LIMA, SHIBATA AND ATALLA [1], have only been possible by the use of activation analysis. If classical analytical chemistry methods had been used, samples of about one gram would be required

for each analysis. However, using the technique of activation analysis, the mass of samples can be set to about one milligram. Besides, the method is such that various samples of blood, urine, hair, nails can be analysed in a very short time; classical methods would require more than one chemist and the time for all duplicate or triplicate samples, and for all variety of samples, would be extremely long, making the work almost impracticable.

The same technique has been largely used, in various laboratories, in other general problems of Forensic Medicine, where an availability of large amounts of samples is not always possible. Quite often less than milligrams of the sample have to be used for detection and analysis of elements present in proportions corresponding to the levels of parts per million when not parts per billion. With progress made in the methods and techniques of activation analysis the problem of analysing very small samples has been solved. In a very good number of cases it is possible to carry out the analysis instrumentally, without chemical destruction of samples which can be preserved for further presentation in court, as evidence, if such should be the case.

EXPERIMENTAL

Trying to make a distinction between arsenic present in hair as a consequence of excretion and the arsenic externally deposited, various ways and techniques of washing were tried using samples purposely contaminated with arsenic dust or arsenic solution.

Normal hair, taken from healthy people (female) were kept in contact, in agitation, with arsenic trioxide dust for various days. The same procedure was applied for sodium arseniate solution.

After the time required for the experiment considered finished, the hair sample was removed from the flask, where it had been kept in contact with arsenic dust (or arsenic in solution), and excess of dust was removed with a camel hairbrush; samples which had been kept in solution received a slight washing with deionised water.

In order to get samples of hair in which arsenic was present as a consequence of excretion of arsenic swallowed by the patient, injections of sodium arsenite solution, at a concentration of 0.3 to 1.0 mg/ml (in As), were given to a rabbit (female, four kilograms weight), amounting to a total of 67 mg of arsenic in 37 injections in 45 days when the rabbit died. Immediately after death the hairs, grown during the arsenic injections, were taken for analysis. These hairs had grown from spots in which the hair had been removed with a razor before starting the injections. Analysis for arsenic were made in both samples, those taken before application of arsenic, and others taken after the rabbit's death.

In this way three kinds of samples became available: those for which it was known that arsenic was present as a consequence of external contamination; the rabbit's hair, in which arsenic was presented as consequence of a normal metabolic process, i.e. excretion and finally, hair from workers in arsenic plants in which arsenic might be present as a consequence of external contamination as well as of metabolic excretion. To the three sets of samples various washing techniques were applied.

Washing of the arsenic present in the hair externally contaminated indicated that the longer the time the hair had been in contact with arsenic dust, or an arseniate solution, the harder would it be to remove the arsenic by a particular washing technique. Arsenic in the form of dust (arsenic trioxide) gave a contamination more difficult to be removed than in case of a contact with arsenic in solution (sodium arseniate). A contact of four and a half days of hair and arsenic dust or an arsenic solution showed that in the

case of dust, hair had already fixed the arsenic; on the other hand, the experiment with the solution showed that the arsenic was not fixed on the hair in the same time.

TABLE I shows the influence of time of contact of hair and arsenic dust, on the fixation of arsenic by the hair. Before irradiation of the hair, for analysis, the samples were washed three times with a mixture of acetone-methanol 1:1, for five minutes each washing, followed by a three times washing with deionised water, five minutes each washing, also.

TABLE I

Influence of contact time of hair with As_2O_3 on the amount of arsenic fixed on the hair

Contact time (days)	As (ppm)
10	610
38	630
60	1 060
76	1 250

Hair contaminated with the sodium arseniate solution at 10% fixed the arsenic less strongly than when in contact with arsenic oxide dust. After 26 days of imersion in an arsenic solution an amount of 124 ppm of arsenic was found in the hair, after the same washing procedure with the acetone-methanol mixture followed by deionised water.

In order to see if hair externally contaminated would behave the same way as the worker's hair, as far as washing was concerned, the same washing technique was applied to the two types of hair. In this case washing was carried out by maintaining the hair samples in agitation with water, at a room temperature, for various periods of time. Washing by soxhlet extraction, with water, was also applied, in both cases. In this last experiment the corresponding water temperature was 76°C. Results are presented in TABLE II.

TABLE II

Arsenic still present in hair after water washing

Washing time (days)	Arsenic (ppm)	
	Worker	Externally contaminated
0	290	18,000
7	129	136
12	96	100
14	90	91
0.5*)	66	70
0.8*)	60	70

*) Soxhlet washing, 76°C.

In this washing experiment the initial amount of arsenic in the worker's hair and the one externally contaminated were 290 ppm and 18,000 ppm, respectively.

The behaviour of a hair sample in which arsenic was present as a consequence of the metabolic excretion, concerning washing, was verified using a rabbit's hair. Results for this type of hair and comparison with the worker's hair and those externally contaminated are presented in TABLE III.

TABLE III

Effect of washing on three types of hair

	As (ppm)		
	Original	Ether*)	Water**)
Rabbit.....	75	75	27
Worker.....	460	458	76
Externally contaminated	1,070	1,012	47

*) 9 hours soxhlet extraction.

***) 15 hours soxhlet extraction (76°C).

Various other solvents were used for washing of the three types of hair, ionic and non-ionic: water alcohol mixture 1:1, ammonium carbonate 1%, sodium carbonate 1%, acetic acid 2%, triethanolamine with oleic acid, fatty alkylol amide condensate (coconut and peanut oils, denominated detergents 745 and 55 respectively), sodium lauryl ether sulfate (640 detergent) e lauroyl cycloimidinium 1 ethoxy ethyonic acid-2 ethyonic acid disodium salt (detergent 412). With all only a partial washing was achieved and the results were not reproducible.

The best types of washing material, as far as reproducibility was concerned, were water at room temperature or in soxhlet extraction at 76°C, acetone-methanol 1:1 mixture and ether in soxhlet extraction.

Ultrasonics in conjunction with water, acetone-methanol 1:1 mixture, carbon tetrachloride and diethyl ether was also tried, with no better results than the solvent without ultrasonics, as far as arsenic removal is concerned.

All the washing procedures described have been applied before irradiation of the hair sample. In order to see if different results would be obtained if the hair was first irradiated and then washed, the experiment was carried out using the same washing techniques as described before.

In the case of the workers the same results were obtained whatever the order of irradiation or washing, i.e., irradiating and washing or washing and irradiating.

However, for the hairs which had been contaminated purposely, externally, removal of arsenic, if washing was applied after irradiation, was much more difficult than if washing preceded irradiation. A sample, externally contaminated, with 21,000 ppm of arsenic was submitted to washing with water, for 15 hours, in a soxhlet extraction (76°C) and next irradiated and analysed, giving the result of 50 ppm. However, when another sample of the same hair, with the initial amounts of arsenic corresponding to 21,000 ppm was first irradiated and then submitted to the same washing procedure as before, the result was 1,300 ppm of arsenic. After 45 hours of washing the amount of arsenic still was of 650 ppm. The same behaviour was observed if, instead of water, detergents 55, 640 and 412 were used, i.e., removal of arsenic with the detergents was more difficult after the hair had been irradiated.

The results of analysis for blood, urine, nails, hair roots, head and pubic hair, of the arsenic plant workers are presented in TABLE IV.

TABLE IV

Worker	As (ppm)					
	Blood	Urine	Nails	Hair roots	Head hair	Pubic hair
No. 6	0.20	0.05	309	36	220	527
No. 10	0.30	0.10	456	80	586	611
No. 11	0.20	0.10	565	64	593	1,012
No. 12	0.40	0.08	340	126	235	315
No. 13	0.20	0.10	623	94	212	392
No. 14	0.20	0.15	374	56	214	428
Normal values	0.09	0.013	0.52	--	0.51	---
	^a 0.50	^a 0.33	^a 5.60		^a 2.10	

From TABLE IV it is to be seen that the amount of arsenic found in blood and urine are normal but above normal for nails, and head hair.

As far as variation of concentration of arsenic along the hair length the same general trend is observed for the hair of all arsenic plant workers, i.e., there is a maximum of concentration between one and three centimeters, from the scalp end, and then the concentration decreases gradually to the distal point.

In order to see if the distribution of arsenic in the nails of the workers was uniform or not, various nail clippings, taken from the same person, were analysed. The original sample was first scraped with a razor, washed with the acetone-methanol 1:1 and then with deionised water. For four fractions of the nail the following values were found: 302, 180, 510 and 350 ppm. The external and internal parts of the nail, as obtained from the scrapings, gave the values of 707 ppm for the external part and 540 ppm for the internal part. The latter one was next submitted to the same procedure of razor scraping giving 437 ppm for the external part and 610 ppm for the internal.

ANALYTICAL PROCEDURE

When the hair samples had a high amount of arsenic, from 70 ppm and above, analysis could be carried out by purely instrumental technique, using multichannel analysers. Less than 70 ppm of arsenic was difficult to analyse without chemical processing of the sample.

For these samples, with less than 70 ppm, a mass corresponding to about 50 milligrams was irradiated for six hours in a thermal neutron flux of about 10^{12} neutrons per second and per square centimeter. To the irradiated sample, carriers for arsenic and for antimony were added, 50 mg of each. The sample, and carriers, were treated with 2 ml of hydrogen peroxide at 130 volumes, 5 ml of sulfuric acid $d=1.84$ g/ml, 10 ml of nitric acid $d=1.40$ g/ml and heated. Nitric acid was added to the point when the solution became transparent and when white fumes was formed. The solution was transferred to a distilling

apparatus, 40 ml of hydrochloric acid $d = 1.19$ g/ml and 2 g of potassium bromide were added and the arsenic was distilled at 104°C as arsenic trichloride and received in hydrochloric acid. After the arsenic had been eliminated the antimonium trichloride distilled at 110°C .

The arsenic that had been received in hydrochloric acid was transferred, with water, to a beaker and 3 g of sodium hypofosfite were added. The mixture was heated for half an hour until formation of a precipitate of elementary arsenic. The precipitate was filtered and its activity was determined in a 400 channel analyser at the peak of 0.55 Mev. The same procedure was used for standards of arsenic.

CONCLUSIONS AND DISCUSSION

Concerning the time of contact of arsenic and hair, in the cases of external contamination, it is seen from TABLE I, that the longer this time of contact is the harder the removal of arsenic from hair, whatever is the washing procedure used. It seems that arsenic reacts progressively and irreversibly with keratin and that irradiation accelerates the reaction, what is shown by the larger difficulty in washing the hair, as far as arsenic is concerned, after irradiation, when compared to the washing before irradiation. It is possible that the bonding of sulphhydryl groups on keratin are broken on irradiation and arsenic is then incorporated by one of the free bonds.

Comparison of washing effect on the worker's hair and hair contaminated "in vitro" is shown in TABLE II. If the removal of arsenic from hair by water washing is considered on a percent basis it is seen that around 99% of removal of arsenic is attained by water washing at room temperature or at 76°C , for the hair externally contaminated; on the other hand, the same washing procedure applied to the worker's hair gave 69% and 79% removal for washing at room temperature and for washing at 76°C . It seems that in the case of external contamination a practically total removal of arsenic (99%) was achieved by these water washing procedures, although the hair still has 70 ppm of arsenic. With the worker's hair the same amount of about 70 ppm is reached after the washing and this still represents around 30% of the original amount. The value of 70 ppm may be a saturation value for arsenic eventually linked to the keratin. Consequently, it is shown that it is not possible to differentiate arsenic present in the hair by external contamination or by metabolic excretion by using water washing procedures for this aim.

The initial amounts of arsenic in hair contaminated "in vitro" (externally) and in the worker's hair are 18,000 and 290 ppm, respectively. Since those contaminated "in vitro" will have a large amount of arsenic physically deposited on the hair and not chemically combined to the keratin it will be very easy to remove a large percentage of the arsenic by washing. For the worker's hair, since arsenic and hair have been in contact for quite a long time (months, at least), it is possible that the 70 ppm limit value of saturation has already been reached. For the "in vitro" contaminated hair the same saturation value of 70 ppm may, also, already have been reached; however on a percent basis, this is a very small fraction of the 18,000 ppm initial amount of arsenic.

That the arsenic is already linked to the keratin, at the saturation level of 70 ppm, in the case of the workers, is shown by the behaviour, concerning washing, of worker's hair washed after or before irradiation. No difference is observed on the easiness of washing for the irradiated and non irradiated worker's hair, as well as on the amount of arsenic that remains after washing, which is 70 ppm, for both, whether washing is carried out prior or after irradiation.

However, for hair contaminated "in vitro", and for which most of the arsenic was not completely linked to the keratin, irradiation will give place to the linkage, and washing before or after irradiation will, consequently, be different, being easier to wash before irradiation, when the arsenic was only deposited on the hair, but not chemically bound, than after irradiation, when linkage had taken place.

The same experiments, concerning washing with water, carried out with rabbit's hair, have shown that even the arsenic metabolically excreted is partially removed on washing with water, TABLE III.

Washing with sodium hydroxide is very efficient in all three cases (contamination "in vitro", worker's hair and rabbit's hair), but there is a visible destruction of the hair structure.

All other solvents used, and mentioned previously, gave only partial removal of arsenic and without reproducibility.

The data presented and discussed in this paper indicate that arsenic externally deposited on the hair and arsenic metabolically excreted by the hair cannot be differentiated by washing techniques.

The amount of arsenic in the blood and urine of arsenic plant workers is normal (TABLE IV) and does not indicate that the high proportion of arsenic in the worker's hair, as well as on other keratinic tissues, is a consequence of metabolic excretion. However, one must bear in mind that blood and urine are only a via of excretion which do not, by themselves, concentrate arsenic as the keratinic tissue do.

If all of the arsenic present in the worker's hair was excreted by metabolism, one should expect a more uniform distribution of arsenic along the hair length, since the workers were exposed to constant concentration of arsenic in the air inhaled. Lack of homogeneity of arsenic distribution along the hair length indicates that the arsenic had not reached the hair roots by the blood supply. The higher concentration of arsenic in between the first and the third hair centimeter, starting from the scalp, seems more to indicate that the washing is less effectively carried out at this portion of the hair, than at the scalp or distal point.

Lack of homogeneity of arsenic in the worker's nails suggests also external contamination rather than metabolic elimination of arsenic through the nails.

The amount of arsenic present in the worker's hair roots, where external contamination is not probable, is, on the average for the six cases examined, 76 ppm, a value which is very close to those found for hair when washing has removed arsenic not linked to the keratin.

With the amount of data accumulated up to now it seems that although the arsenic plant workers are daily exposed to abnormal amounts of arsenic dust and that they forcedly inhale or ingest a very high percentage of the dust, most of the arsenic is eliminated. However, no data have been obtained concerning the concentration of arsenic in the different organs such as liver, kidney, lungs, etc., after death. Only an activation analysis would decide if the continued inhalation or ingestion of arsenic by such workers could have created a sort of mithridatism in which the labourers may have acquired a certain immunity to arsenic.

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RESUMO

Estuda-se neste trabalho o problema da presença de arsênico, em altas proporções, nos cabelos de trabalhadores de usinas de arsênico, procurando identificar se esta grande quantidade daquele elemento resulta de uma contaminação externa ou se resulta de excreção do arsênico ingerido cotidianamente, pelos operários, na forma de pó. São também apresentados os resultados das análises de arsênico nas unhas, sangue, urina, cabelos da cabeça e do pubis, daqueles operários.

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