degree of the contractions and relaxations can be so controlled that the origin and insertion of the muscle need not be approximated until desired; consequently, the beneficial physiological changes called into activity by muscle action are produced with the minimal amount of strain of its fibres, and the movements of the injured joint as a whole can be kept within minimal range. If treatment is based on these principles no uncomplicated acute sprain should become a chronic one.

ARSENIC IN ITS RELATION TO THE KERATIN TISSUES

BY

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The keratin tissues, hair, nails, and skin, are of considerable importance in connexion with arsenic poisoning from a physiological as well as from a medico-legal point of view. In many of the published cases the presence and distribution of arsenic in these tissues has been of major significance in the investigation, and it seemed worth while, therefore, to obtain further experimental information on certain points concerning the effects of arsenic on the keratin tissues.

It is obvious that a knowledge of the mechanism of arsenic fixation in hair may give an important insight into the body's defence against the poison, and that examination of hair in certain cases of arsenical poisoning may be expected to yield information of great value. From the medico-legal aspect it would seem that the finding of arsenic in the hair would be strong evidence of its administration; but before the analyses may be so interpreted it is necessary to inquire into a number of other possibilities. For example, is it possible for hair to absorb arsenic from fluid bathing the shaft; or does any arsenic present necessarily come from the body fluids bathing the hair root? The importance of such a question may be illustrated by reference to one or two cases.

In the Seddon case the victim died about fourteen days after the first dose of arsenic, and this element was found not only in the proximal parts (3 mg. per 100 grams hair), but also in the distal parts of the hair (2 mg. per 100 grams hair), which was about twelve inches long. The presence of arsenic in the distal three inches raised the presumption that arsenic had been administered more than a year before death. Willcox suggested, however, that the arsenic in the distal ends might have been absorbed from the blood-stained body fluid which had come into contact with the hair, and by *in vitro* experiment proved that this explanation was possible. *Per contra*, in the Hearn case, the presence of arsenic in the hair of Miss Everard was interpreted as indicating the administration of arsenic over a considerable period, whereas it may well have been due to contamination from water which had soaked into the coffin after percolation through the arsenic-containing soil. Thus from similar facts we have opposite deductions made on behalf of the Crown, and both to the disadvantage of the accused.

It is well known that arsenic may be found in the hair after all traces of it have disappeared from other organs of the body, but it is desirable to ascertain whether the quantity found indicates a preferential absorption of

arsenic by the keratin tissues or merely a trapping of arsenic in the non-vascular tissues. It seems further of interest to ascertain whether, if there is preferential absorption, it is a function of living pre-keratin tissues or whether keratin itself absorbs arsenic preferentially *in vitro*. It is also desirable to know if this absorption is due to chemical combination between arsenic and certain products of cellular activity, or if it is a case of simple absorption. Other problems include those of ascertaining whether or not arsenic can travel along the hair shaft; whether it is possible to ascertain from the distribution of the poison if it has been absorbed from the body or if it has resulted from external contamination; and whether any inferences of value can be drawn from the amount and distribution of arsenic as to the time when a dose of arsenic was administered, and the size of the dose. We are still able to answer very few of these questions, and the present paper must be looked upon as a preliminary report.

Preferential Absorption

If we consider the results of a series of cases dealt with in the report of the Royal Commission on Arsenic Poisoning (1904) we find that the keratin tissues do take up more arsenic, weight for weight, than the other tissues of the body. For example, a number of individuals were given 5 mg. of arsenic per day for two months; on examination the hair of these men was found to contain from 2 to 5 mg. of arsenic per 100 grams of hair. The total quantity of arsenic taken by each individual was approximately 300 mg., and if this amount, without allowing for excretion (which would account for the greater part of it), be supposed to be equally distributed over the body the concentration would be about 0.5 mg. per 100 grams of body weight—that is, about one-tenth of the quantity actually found in the hair. Similar figures can be shown for the nails and to a lesser extent for the skin.

(A) BY HAIR REMOVED FROM THE BODY

It is known, as has been mentioned, that hair soaked in an arsenical solution absorbs arsenic, but it is evidently desirable to place this observation on a quantitative basis and to ascertain whether, under such conditions, the hair can attain to, or surpass, the concentration of arsenic in the surrounding fluid. For this purpose a series of tubes were set up each containing 0.5 gram of hair or nail clippings and 10 c.cm. of a solution of sodium arsenite containing 0.01 mg. of arsenic per c.cm. The liquid was decanted from each at definite intervals, and an aliquot was analysed by the Marsh process. The hair in each case was removed and washed with six portions of water, and allowed to drain thoroughly after each washing. The organic matter was destroyed by the method of Wood-Smith¹ of boiling with equal parts of concentrated nitric and concentrated sulphuric acids. After complete removal of nitric acid the liquid was made up to a known volume, of which an aliquot was boiled with 0.5 gram of potassium metabisulphite to remove the last traces of nitric acid and reduce arsenate to arsenite. Arsenic was then determined by the electrolytic Marsh apparatus. The results of one typical experiment of the series are shown in Table I, where the density of human hair is taken as unity. Since, however, part of the absorbed arsenic might conceivably be due to swelling of the hair, a known weight of hair (0.3036 gram) was allowed to soak in water for seventy-two hours; at the end of this time it was removed and dried as thoroughly as possible with filter paper in the same way as the arsenic-containing hair (it still felt damp to the touch). The increase in weight was 0.0221 gram, or 7.3 per cent. When this hair was allowed to stand in

the balance case for half an hour the increase fell to 0.0040 gram, or 1.3 per cent. In other words, there was no simple imbibition of the fluid surrounding the hair, and the figures of Table I represent a true absorption of arsenic.

TABLE I

Length of Time of Soaking	Concentration of Arsenic in the Hair	Concentration of Arsenic Left in the Solution	Percentage of Arsenic Recovered
	mg. per c.cm.	mg. per c.cm.	
0 hours	0.00	0.010	
20 "	0.024	0.009	102
44 "	0.03	0.008	95
118 "	0.05	0.007	95
145 "	0.05	0.008	105
234 "	0.05	0.007	95

It is to be observed that the hair absorbs arsenic in increasing amounts, gradually attaining a concentration of arsenic about seven times that of the surrounding solution. Evidently, too, the final concentration is greater than the usual figure hitherto reported; it is unaltered by increasing the time of soaking above seventy-two hours; and generally, it is not increased when the hair is soaked in a stronger solution of arsenite. In the cases given in the Royal Commission Report, the level of arsenic in the hair runs fairly uniformly at about 2 to 3 mg. per 100 grams hair, not only in those cases where small doses have been given over a long period but also in those where the hair has been examined long periods after a single dose.

TABLE II

Quoted by	Reference	Mg. Arsenic per 100 grams Hair	Notes
Casper-Liman	<i>Gericht Med.</i> , 8th ed., ii, 378	2.0	—
Bruuardel ...	<i>Ann. d'Hyg.</i> , 1899, xxii, 137	1.0 2.5	Subacute
Knecht and Deardin	<i>Lancet</i> , 1901, i, 854	1.0 3.0 3.0	Chronic
Heffter ...	<i>Arch. Inter. Pharm.</i> , 1905, xv, 399	1.0	59th day after one dose of about 1 gram
Althausen and Gunther	<i>Journ. Amer. Med. Assoc.</i> , 1929, 2002	1.5 2.7 1.8 (beard)	2.5 months after a single dose 3.5 " " 3.5 " "
Royal Commission Report on Arsenical Poisoning, 1904, 336		3.0 3.0 3.0 1.85 1.5 1.5 0.75 0.75 0.30	2 months after dose " " " " " " " " " " " " " " " "
Bannister ...	<i>British Medical Journal</i> , 1920, ii, 476	5.0	4 days after inhalation of arsine
Seddon case...	—	Proximal—3.0 Distal —0.2	Probably 13 days from the first dose
Armstrong case	—	0.45	Probably 8 days from the first dose
Hearn case: Mrs. Thomas	—	0.7	17 days after the first dose
Miss Everard	—	1.5 in. from scalp—2.3 1.5 in. " middle—1.5 1.5 in. " distal—1.0	Suggested 7 months from the first dose

In all the published cases of arsenic poisoning in which the hair has been analysed the level of arsenic concentration runs about the same figure. (Table II.)

(B) BY NAILS REMOVED FROM THE BODY

In the case of nails the original weight of 0.330 gram increased by 38.2 per cent. after 144 hours' soaking. After standing three hours in the balance case, this increase

was reduced to 17.6 per cent. Before soaking in arsenic solution the nails were scraped as free as possible from adhering skin, and before analysis the liquid was removed by filtration and thorough washing with distilled water. Results obtained are shown in Table III, where the density of human nail is taken to be 1.2. The last column represents the concentration of arsenic in the nails corrected for uptake of the medium on the assumption that 0.5 gram of nail takes up 0.19 c.cm. of the solution (that is, 0.0019 mg. of arsenic and 38 per cent. of its volume of the solution).

TABLE III

Length of Time of Soaking	Concentration of Arsenic in the Nails	Concentration of Arsenic left in the Solution	Percentage of Arsenic Recovered	Concentration of Arsenic in the Nails (corr.)
	mg. per c.cm.	mg. per c.cm.		mg. per c.cm.
0 hours	0.000	0.010	—	0.000
67 "	0.084	0.007	105	0.079
93 "	0.084	0.006	95	0.079
116 "	0.107	0.007	115	0.103
188 "	0.192	0.0018	98	0.185

It is a remarkable fact that nails in arsenical solution can take up arsenic to a concentration of about 100 times the final concentration of the surrounding solution, and in our experiments had not even then reached the maximum.

Evidently both nails and hair removed from the body and soaked in a solution containing arsenic (as sodium arsenite) can absorb that element until the concentration is far in excess of that of the surrounding medium. In other words, they exhibit preferential absorption. That this absorption is not merely a case of surface adsorption is shown by the fact that when hair is soaked in sodium arsenite solution, as in the above experiments, none of the arsenic is removed by thorough washing with dilute caustic soda before analysis. The importance of this observation can hardly be exaggerated, for it has been customary to consider a higher concentration of arsenic in the keratin tissues than in the viscera as proof that the arsenic was absorbed by those tissues during life; that assumption is proved to be false.

Absorption from the Living Body

Under the conditions of our experiments the arsenic is evenly distributed along the length of the hair, but if arsenic is being absorbed into growing hair or nails from living body fluids, a different distribution is to be expected. At first arsenic should be found only in the proximal end. As the hair grows either an arsenic-containing band grows with it (in the case of the poison being administered over a short period) and is followed by an arsenic-free proximal zone, or, in the case of long-continued administration or continued presence of arsenic in the blood owing to slow excretion, more and more of the hair contains arsenic until, eventually, through cutting or falling, the distribution becomes uniform.

In the majority of cases of poisoning one would expect to find more arsenic in the proximal end of the hair than in the distal, but, of course, for a variety of reasons, this distribution is not absolute. For one thing, the relative lengths of the arsenic-containing and arsenic-free parts will vary from case to case with the time of administration of the poison, the rate and amount of excretion in the vomitus, urine, and faeces, the rate of growth of the hair, and the time and frequency of hair-cutting. Moreover, as Willcox has pointed out, "sweat and sebaceous secretion contain arsenic, and as the whole length of the hair comes in contact with these secretions, the limitation of arsenic to the proximal portions of the hair is relative,

not absolute." However, in cases of absorption *in vivo* some such distribution may generally be expected, and the finding of more arsenic in the proximal parts than in the distal is strong evidence that the arsenic has been absorbed from body fluids—*provided it can be shown that no external contamination can account for it*. The expectation of a non-uniform distribution of arsenic in hair is in any case dependent on the supposition that the arsenic, once absorbed into the hair, remains fixed and can neither travel along the shaft nor be reabsorbed by the body, and experiments to justify this supposition have hitherto been lacking.

Methods of Absorption Determined

The following experiments were carried out *in vitro* to determine whether or not arsenic is capable of travelling along a hair fibre. A lock of human hair about 10 cm. long was placed so that the cut ends dipped to the extent of about 4 cm. into a solution of sodium arsenite containing 0.1 mg. of arsenic per c.cm. This solution was contained in a test tube and the hair was arranged round a tightly fitting cork so that 5 cm. of it was completely out of contact with the fluid. Within twenty-four hours it was observed that the hair above the stopper was damp for a length of 2 cm. It was allowed to stand thus for three weeks, and at the end of that period the hair projecting above the stopper was divided into five sections, and these were analysed without previous washing. The results of the analyses are shown in Table IV.

TABLE IV

Weight of the Section	Total Amount of Arsenic Present	Notes
0.144 gram	0.5 mg.	Section nearest the solution. Very wet
0.122 "	0.15 mg.	Hair damp
0.104 "	Trace	Slightly moist in one part
0.100 "	Nil	Hair quite dry
0.067 "	Nil	Section farthest from the solution. Dry

Two important points emerge from these results. First, the hair not immersed in the liquid but wetted with it by capillary action actually contains more arsenic than the hair in contact with the solution. This is due to evaporation of the arsenical fluid on the hair fibre. Secondly, local contamination at the proximal end has resulted in that gradual distribution which is to be expected if absorption takes place from the body fluids. But it is evident that it may equally occur after death if by any chance the hair is soaked in an arsenical solution at the proximal end only.

In order to prevent the solution travelling up the outside of the fibre, the experiments were repeated with a thin layer of paraffin wax laid round the top of the cork. The hair was allowed to stand for three weeks in a solution containing 0.1 mg. of arsenic per c.cm. No moisture was visible outside the tube and the volume of the solution did not apparently diminish; 3 cm. of the hair (weighing 0.164 gram) nearest the liquid contained no arsenic.

The experiments indicate beyond doubt that local wetting of the hair shaft with arsenite solution causes arsenic to be absorbed over a length of hair by "creeping" of the solution and absorption from outside. They show equally definitely that travel of the arsenic along the inside of the fibres does not take place, and that, therefore, the graduated distribution of arsenic is legitimately to be attributed to *in vivo* absorption only if contamination from some external liquid source can definitely be excluded.

Reabsorption by the Body

They also suggest that arsenic once deposited in hair or nails is finally lost to the body—a suggestion which is *a priori* indicated by the fact that hair shafts, the keratin layers of epidermis, and so on, are really dead tissues, and are, indeed, excretions. However, there appears to be a division of opinion on the subject, for whereas Willcox states that "it is probable that during the prolonged administration of arsenic a certain amount becomes deposited in the skin, hair, and nails, and this is gradually reabsorbed, being excreted by the urine and faeces," Althausen and Gunther,² on the basis of a study of excretion of arsenic before and after treatment with sodium thiosulphate, have shown that arsenic is not reabsorbed from the hair.

In our early experiments the fact that we were able to rinse arsenic-soaked hair several times with water and even with dilute caustic soda without appreciable loss of arsenic suggested that reabsorption of arsenic from hair to the living body was at any rate a slow and difficult process. Quantitative experiments on this point, however, showed that arsenic-soaked hair containing the maximum of 5 mg. per 100 grams could lose part of the arsenic after prolonged soaking in distilled water. After fifteen days' soaking in this manner, the water being changed every twenty-four hours, about 40 per cent. of the arsenic had been removed. Further soaking would not remove the other 60 per cent. Furthermore, it was found that the arsenic could not be removed to any appreciable extent from the hair of a person who had been receiving arsenical injections. This suggests a method of differentiating between arsenic absorbed *in vivo* and arsenic derived from some external source of contamination.

Summary

It has been shown that keratin tissues when soaked in arsenical solution exhibit the phenomenon of preferential absorption. The arsenic so absorbed can be partly removed by very prolonged soaking in distilled water, but rapid rinsing either with water or dilute alkali does not affect the arsenic content in any marked degree. By this method the maximum arsenic content of hair is found to be 5 mg. per 100 grams, and much higher values can be obtained in the case of nails. *In vitro* experiments have shown that arsenic can travel along a hair fibre only if "creeping" of the solution along the outside of the hair be possible. It has been demonstrated that arsenic absorbed into hair *in vivo* cannot be removed by prolonged soaking, and this fact is tentatively put forward as a basis for a method of distinguishing between arsenic absorbed *in vivo* and arsenic resulting from external contamination.

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REFERENCES

- ¹ Wood-Smith: Royal Commission Report (1904), ii, 351.
- ² Althausen and Gunther: *Journ. Amer. Med. Assoc.*, 1929, 2002.

The *Transactions* of the Twentieth Annual Conference of the National Association for the Prevention of Tuberculosis have now been published. This conference was held last June, and was reported at the time in these columns. It was largely concerned with reviewing the progress made in the twenty-one years of the life of the National Association, and this volume of *Transactions* has consequently a definite historical interest. The National tuberculosis scheme is discussed from various points of view, and a comprehensive survey of the present position is thus given. The main trends in further development are indicated.