In applying this hypothesis it was assumed that allowance was necessary for the influence of aggregation on the cohesive constant A of the equation of state. The assumption made was that A for the aggregated species was twice per mole the value of the unaggregated species. The aggregation equation was found to be, where y is the aggregated fraction, $\log_{10}(v - \delta)y/(1 - y)^2 = 527/T - 1.25 \log_{10}T + 0.813$. The pressures are accurately represented by the equation of state, wherein allowance has been made for aggregation.

5. The pressure-volume products, as functions of reciprocal volume, are given in series form for each of the five temperatures measured. The equation of state 4′, however, represents the entire range of data with satisfactory exactness.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY OF THE NORTH DAKOTA AGRICULTURAL COLLEGE]

A HITHERTO UNSUSPECTED SOURCE OF ARSENIC IN HUMAN ENVIRONMENT

BY ROE E. REMINGTON

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The question as to whether or not arsenic is a constant constituent of the animal organism has been one of interest since the work of Gautier and of Bertrand. These workers maintained that it is always present, at least in man, one of them going so far as to assert that it exercises a normal and necessary function in the body. This position has been attacked by Ziemke and others, who maintain that arsenic is present only as an accidental contamination from many articles of food and drink, and may be, and oftentimes is entirely absent in the body. Before we can interpret the work of any investigator, however, we must scrutinize his methods and technique, and failure to find arsenic with certainty cannot in all cases justify the conclusion as to its absence.

The literature of this phase of the question has been fully summarized by Fordyce, Rosen and Myers,[1] and will not be recapitulated here. By far the most painstaking and delicate work of recent times has been that of Billeter and Marfurt,[2] who examined the organs and tissues of many bodies, from new-born infants to men of seventy years. They have so improved the Marsh method as to detect and estimate with certainty quantities of arsenic as small as one or two millionths of a milligram. Their conclusion is that arsenic is always present and progressively increases with age. They have established that in round numbers the body of an adult contains one ten-millionth of its weight in arsenic.

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[1] Fordyce, Rosen and Myers, *Arch. Internal Med.*, **31**, 739 (1923).

[2] Billeter and Marfurt, *Helv. Chim. Acta*, **6**, 258, 771, 780 (1923).

The arsenic of the body is usually attributed to food, (1) because of chemical treatment in manufacture, (2) taken up by plants from the soil, or (3) present in sea food. Headden[3] reported that our virgin soils contain from 2.5 to 5 parts per million, the underlying marl 4 to 15 parts, and that crops grown on such soils, and animals fed on such crops contain arsenic. Likewise, Reichert and Trelles[4] found arsenic in all of 20 samples of soils from Argentina, while Bado and Zanetta[5] reported traces in the water from a number of deep wells in the City of Buenos Aires. Bertrand,[6] in a review of French literature, cites the amounts present in various foods and concludes that arsenic is a constant constituent of plants and animals. Fish have been reported to contain three to four parts per million, and the urine of fish eaters is said to contain a higher percentage of arsenic than that of other individuals. Arsenic in foods through chemical treatment has been familiar since the time of the Manchester beer cases, through the use of impure acid in making glucose. Although the maximum amount of arsenic found in any of the samples of beer analyzed was only four parts per million, hundreds of persons were made ill, and numbers died.[7] As long ago as 1913 the North Dakota Food Commission set up one part per million as the maximum permitted in baking powder. Within the last year or two the British Ministry of Health has fixed one one-hundredth grain (as arsenic trioxide) per pound[8] as the amount permissible in American apples, and the United States Department of Agriculture, while not disposed to establish a rigid limit, does not consider the British requirement as unduly severe. The widespread use of arsenical sprays in America has an important bearing on this matter, not alone from the point of the arsenic mechanically carried, but that absorbed by the plant as well.

It is the purpose of this paper to call attention to a heretofore unsuspected potential source of a part, at least, of the arsenic in the human body. Fordyce, Rosen and Myers[1] examined a large number of samples of so-called normal human blood and urine, and often were not able to account for the amount of arsenic which they found, except to explain it by the general statement that it came from dietary sources. Elimination is not always a guide as to the amount of arsenic actually present in the body, as Myers and others[9] found when they administered sodium thiosulfate in certain skin affections. Since Federal and State regulations regarding foods are rather strict, and have been in force for some years,

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[3] Headden, *Proc. Colo. Sci. Soc.*, 9, 345 (1910).

[4] Reichert and Trelles, *Anales asocn. quim. Argentina*, 9, 89 (1921).

[5] Bado and Zanetta, *ibid.*, 9, 24 (1921).

[6] Bertrand, *Bull. soc. hyg. aliment.*, 8, 49 (1920).

[7] Witthaus, *Manual of Toxicology*, William Wood and Co., New York, **1911**, p. 419.

[8] 0.01 grain per pound = 1.207 mg. per kg.

[9] Myers and others, *J. Lab. Clin. Med.*, **11**, No. 9, June, 1926.

it is difficult to imagine a daily elimination by kidney alone of several tenths of a milligram as coming from diet.

The writer has examined a large number of brands of manufactured tobacco, in the form of pipe tobacco, cigars, cigarettes and chewing tobacco, and has found arsenic to be invariably present in quantities many times greater than the amounts cited as being permitted in foods. Spallino[10] has reported analyses of three samples of Italian snuff and four samples of Italian smoking tobacco containing arsenic, but from the abstract of his paper available to me I do not gather that he made any experiments towards determining what becomes of the arsenic when the tobacco is used.

The method used in my work for the separation and estimation of arsenic is a micro-Marsh method which has been in use since 1913, and which is capable of separating and estimating by comparison quantities as small as one micro-milligram, or one part in ten million on a 10g. sample.

Organic matter is destroyed by wet combustion, using for this purpose Coors evaporating dishes 16 cm. in diameter covered with watch glasses which fit inside the dish and are placed bell side up. The digestion is started on the water-bath with nitric acid, the sample being moistened with water to prevent ignition. When largely liquefied, 10 cc. of sulfuric acid is added and the digestion continued, first on the water-bath and later on an air-bath with a temperature of 200° *in the bath.* Nitric acid is added as needed from time to time to keep the space beneath the cover glass continuously filled with brown fumes. The material is never permitted to char, and oxidizing conditions are always present. When the digestion is complete, the cover glass is removed and the nitric acid eliminated by repeated additions of water, and heating until a drop of the solution gives no color with diphenylamine reagent. Prior to introduction into the Marsh apparatus the solution is diluted to about 25 cc., heated to boiling and a small crystal of stannous chloride added to insure that the arsenic is in the trivalent state.

The details of the Marshing are not unusual. The generator is started with sufficient 1:4 sulfuric acid to cover the zinc, and allowed to run until action has died down to a practically constant rate and the flame is between 1 and 2 mm. in size, after which the sample is run in at such a rate as not appreciably to increase the flow of gas. Marshing is continued for half an hour after the last of the sample has been introduced. Carefully controlled blank determinations were made from time to time on all reagents. This is important, since the best arsenic-free zinc obtainable gives a distinct mirror under the conditions of the experiment.

Billeter maintains that the last traces of organic matter are not destroyed by wet combustion, and follows it by an alkaline nitrate fusion. I have found that occasionally a sample will froth in the generator, suggesting the possibility of some organic matter being present. It is not claimed, however, that I have recovered the last traces of arsenic, but it is believed that the amounts which have escaped are insignificant.

The amounts recovered are too large to be estimated by comparison with standard mirrors without introducing a large factor, so that the mirrors have been dissolved in standard iodine solution and titrated according to the method of Billeter. The portion of the tube containing the mirror was cut off, dropped into a glass-stoppered 100cc. flask,

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[10] Spallino, *Gazz. chim. ital.*, **43** [II], 475 (1913).

and 25 cc. of 0.002 *N* iodine solution added, followed by 2 cc. of 1% sodium bicarbonate. The stopper was then inserted, covered with a few drops of potassium iodide solution and the neck of the flask covered with an inverted test-tube to prevent evaporation of the sealing liquid. Solution of such large mirrors as those obtained is very slow, requiring sometimes as long as 24 hours. After solution was complete the stopper and neck of the flask were rinsed, and the excess of iodine was titrated with 0.002 *N* thiosulfate solution.

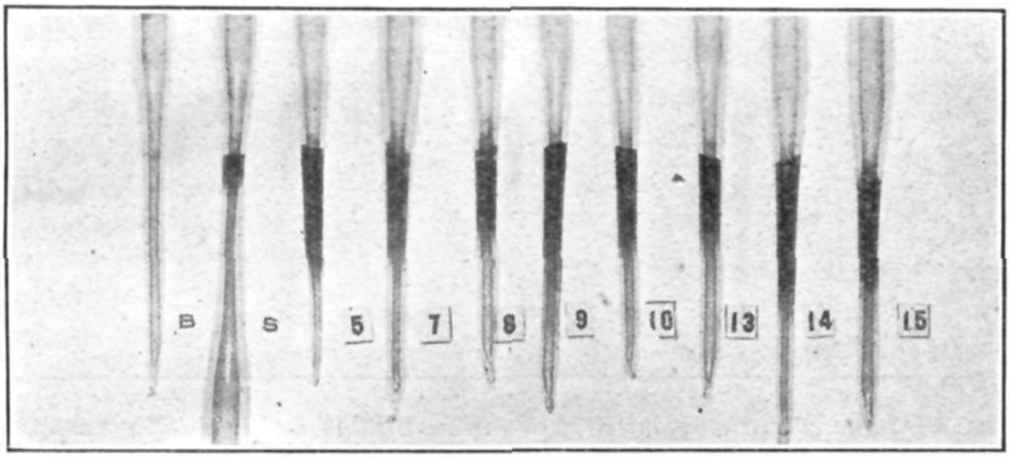

Fig. 1.—Arsenic mirrors from 10 g. of various brands of American pipe tobacco. *B*, blank test on reagents; *S*, standard mirror (10 mmg. of arsenic).

Fig. 1 shows the amounts of arsenic recovered from 10 g. each of a number of pipe tobaccos, shown beside a standard mirror representing

TABLE I

ARSENIC IN AMERICAN PIPE TOBACCO

Sample No.	As, p.p.m.	As_2O_3, grains per pound[8]
2	12.9	0.12
5	14.7	.136
7	12.9	.12
9	16.1	.15
10	14.1	.13
13	6.0	.05
14	28.9	.27
15	17.2	.16

TABLE II

ARSENIC IN PLUG CHEWING TOBACCO

Sample No.	As, p.p.m.	As_2O_3, grains per pound[8]
17	15.8	0.146
18	12.0	.110
19	16.7	.154
20	14.1	.131
21	10.2	.094
22	8.0	.074

TABLE III

EFFECT OF BURNING ON THE ARSENIC OF TOBACCO

Sample No.	A	B
2	8.8	5.2
5	10.5	
9	8.4	13.1
14	13.6	14.5

A, arsenic in parts per million absorbed by sodium bicarbonate solution from smoke.

B, arsenic in parts per million retained in ash of tobacco.

TABLE IV

SOLUBLE AND INSOLUBLE ARSENIC IN PLUG TOBACCO

Sample No.	A	B
16	15.3	13.5
17	6.8	9.1
18	6.1	6.0

A, arsenic soluble in water from plug chewing tobacco (parts per million).

B, arsenic insoluble in water.

one part per million in a sample of the same size. Table I shows the results obtained by titrating these same mirrors. Mirrors of this density cannot be judged at all by the eye, since the intensity as well as the length increases, and the photographs are still harder to judge. Fig. 2 and Table II give the same information for a series of plug chewing tobaccos. Data will not be presented at present covering cigars and cigarettes, although several of these have been examined and the results found to be comparable with those given.

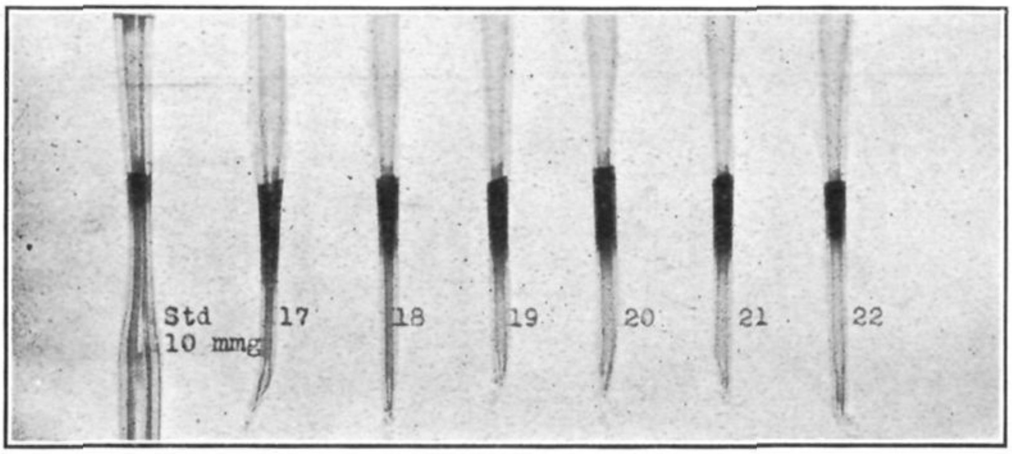

Fig. 2.—Arsenic mirrors from 10 g. of various brands of plug chewing tabacco.

TABLE V

ARSENIC IN VARIOUS VEGETABLE AND FOOD SUBSTANCES

	As, p.p.m.	Reported by		As, p.p.m.	Reported by
Peas	0.04	Bertrand	Beef (muscle)	0.006	Bertrand[a]
Carrots	.05	Bertrand	Veal	.001	Bertrand[a]
Apples	.05	Bertrand	Mackerel	.025	Bertrand[a]
Mushrooms	.06	Bertrand	Grumet (fish)	.06	Bertrand[a]
Pears	.07	Bertrand	Eggs	.08	Bertrand[a]
Rice	.07	Bertrand	Corn stalks	.04	Jadin and Astruc[b]
Potatoes	.08	Bertrand	Corn kernels	.3	Jadin and Astruc[b]
Cauliflower	.08	Bertrand	Dried peas	.26	Jadin and Astruc[b]
Spinach	.09	Bertrand	Dried fruits		
White beans	.10	Bertrand	(sulfur bleached)	0.2–2.0	Collins[c]
Cabbage	.20	Bertrand	Vegetables	0.2	Bang[d]
Lettuce	.23	Bertrand	Fish	3	Cox[e]

[a] Bertrand, *Bull. soc. hyg. aliment.*, **8**, 49 (1920).

[b] Jadin and Astruc, *Compt. rend.*, **154**, 893 (1912); **159**, 268 (1914).

[c] Collins, *J. Ind. Eng. Chem.*, **10**, 360 (1918).

[d] Bang, *Biochem. Z.*, **165**, 364 (1925).

[e] Cox, *Analyst*, **50**, 3 (1925).

The next question naturally raised concerns the fate of this arsenic when the tobacco is smoked or chewed. The device constructed for smoking the tobacco consisted of a tube of hard glass of 12 mm. bore and 25 cm. length, in one end of which was inserted a plug of glass wool, and connected by means of a cork and smaller tube to a nine-bulb absorption tube containing 100 cc. of a 1% solution of sodium bicarbonate. The tobacco (10 g.) was loosely packed into the hard-glass tube and smoked by a water aspirator with medium suction, so that the sample was burned in about an hour. The bicarbonate solution and the ash were examined separately. Analytical results are given in Table III. We can say roughly that half of the arsenic is evolved in the smoke and absorbed by the soda solution.

Solubility tests were made on the chewing tobaccos by soaking 10 g. in about 200 cc. of distilled water overnight, then filtering and washing with an additional 100 cc. of water. Both the solution and residue were examined. Results are shown in Table IV.

Since it may be maintained that the amounts here reported are no greater than are present in many plants which are used as food, data from various sources are appended in Table V.[11]

While these results point clearly to the possibility of a considerable part of the arsenic in tobacco finding its way into the body, it is not felt that they are fully conclusive. Absolute proof must include examination of blood and urine of users and non-users on as nearly as may be identical diet, as well as of organs and tissues of the bodies of persons whose history as regards the use of tobacco is known. This work is now under way. Neither is it felt that the data here presented justify us in drawing any conclusion as to the possibility of chronic arsenical poisoning from the use of tobacco, although such a possibility is not to be dismissed without more evidence. To the toxicologist, who is so often embarrassed by the enigma of "normal arsenic," the knowledge that the habitual user of tobacco is in daily contact with appreciable amounts of arsenic will be of great import.

Acknowledgment should be made of the courtesy of R. O. Baird, North Dakota State Food Commissioner and Chemist, in whose Laboratory the first preliminary experiments were made.

Summary

Samples of American smoking and plug tobacco have been examined for arsenic, and found to contain from six to thirty parts per million, or from 0.05 to 0.27 grain of arsenic trioxide per pound.

Approximately half of the arsenic in pipe tobacco is evolved in the smoke, and about half of that in plug tobacco is soluble in water.

Amounts of arsenic reported are much in excess of the maximum per-

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[11] Since this paper was prepared, Chapman [*Analyst*, **51**, 548 (1926)] has reported the presence of relatively enormous amounts of arsenic in crustaceans. In lobster the average found was 40 parts per million in the fresh meat, but in one sample as high as 110 parts per million were recovered.

mitted by state and Federal authorities in foods, and of the amounts normally present in plants and animals.

FARGO, NORTH DAKOTA

THE INFLUENCE OF WATER ON THE COMBINATION OF THE HALOGENS WITH HYDROGEN

BY MAX BODENSTEIN AND WILHELM JOST

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Recently Lewis and Rideal[1] have reported a research from which they conclude that intensive drying makes impossible not only the combination of hydrogen and chlorine, but also that of hydrogen and bromine and of hydrogen and iodine. They assume further, as a consequence, that all three reactions do not take place between the molecules but that in all these reactions the atoms of the halogens are the actual reacting substances.

We shall discuss not the authors' conclusions, but only their experiments, and shall correct an error which they have made in regard to the old equilibrium measurements on hydriodic acid.

Concerning this last point, they cite a table of 1894 which states that the equilibrium is not independent of the pressure, as required by theory. In doing so, they overlook the fact that this disagreement was the occasion of a thorough investigation[2] in which the basis of the discrepancy was fully cleared up; it lay in an error in the method of analysis used in the first research. In this paper we can add the following table, which was worked out some 15 years ago by Sachtleben for Bodenstein. His object was to show by exact measurements for lecture purposes the independence of the equilibrium from pressure.

Pressure of HI at filling (at 20°)	1 Atm.	2/3 Atm.	1/3 Atm.
Decomposed at 500°, %	23.94	24.01	24.12
Decomposed at 440°, %	21.88	22.06	21.96
Decomposed at 400°, %	20.49	20.67	20.36

These figures are certainly very useful for application in lectures, but their communication in a paper is certainly no longer necessary, for the proof that the decomposition of hydriodic acid obeys the law of mass action was given adequately in the paper[2] of 1897.

As to the probability that a reaction expressed by the equation $H_2 + I_2 = 2HI$, corresponding to an entirely correct bimolecular reaction, goes by means of atoms, we shall say nothing here. However, we shall show that the experiments of Lewis and Rideal are no evidence for this view, that in the intensively dried state the reaction goes differently than in the presence of water vapor.

[1] Lewis and Rideal, THIS JOURNAL, **48**, 2553 (1926).

[2] Bodenstein, *Z. physik. Chem.*, **22**, 1 (1897).