THE FORM IN WHICH BROMINE EXISTS IN THE BODY

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The question of the chemical form in which bromine is present in the animal body is one of great interest. The elucidation is essential for the understanding of the mechanism of action of bromine.

Reports in the literature on this question are contradictory. Some writers consider that bromine is present in organic compounds, while others assert that it exists only in an uncombined, ionic form. For instance, Uhlmann [11] and Zondek and Bier [14] have put forward the view that a bromine-containing hormone is present in the hypophysis (by analogy with the iodine-containing hormone of the thyroid gland, thyroxin), although their claim has not been supported by any experimental evidence. Arima [8] showed that bromine, introduced into the body in the form of KBr, is combined in the liver, kidneys, and brain almost exclusively with the lipids of these tissues, whereas it combines to a very limited extent with proteins. A. F. Shoshin [7] showed that bromine is present in the dog's brain in an ultrafilterable and a non-ultrafilterable form, and that the proportion of the former is greater (33.2%) in the white matter than in the cortex (18.9%), in which more bromine is present in the non-ultrafilterable form. Guillaumin and Merejkowsky [9] showed that between 63 and 88% of the bromine in the blood is present in a non-ultrafilterable form.

Other workers [12, 13] found no protein-bound bromine in the blood. According to Leipert [10], bromine is present in the blood only in the ionic form.

Until recently, therefore, nothing definite was known regarding the chemical form in which bromine exists even in the blood of animals, not to mention the other organs and tissues. A short survey of the literature on this subject until 1937 was given by S. Ya. Kaplanskii [6], and a full review by I. N. Verkhovskaya [5]. All the investigations described above were undertaken by biochemical or physicochemical methods.

We have combined biochemical and physicochemical methods with the method of labeled atoms. As a result of our earlier findings [1-5] and of those described in the present article, we may assume that the problem of the chemical form in which bromine exists has been solved in respect of several organs and tissues.

EXPERIMENTAL METHOD

We used the isotope method, which has many advantages over other methods, of which the most important are its specificity and its high sensitivity. Measurements were made with a type BFL-25 end-type counter, with a counting efficiency of 22%. Preparations with an activity of 100 fissions per minute enabled us to work with gravimetric quantities of the isotope Br^{82} of the order of 10^{-15} g.

The experimental animals (rats, guinea pigs, rabbits) received a subcutaneous injection of a solution of NaBr, labeled with Br^{82}, in a dose of 20-30 μC/kg body weight. Because of this high activity, radiometric estimations of the biochemical fractions could be made. The animals were decapitated 15-20 h after the injection of Br^{82}. Our investigations showed that this time was more than adequate for attaining equilibrium in the distribution of the injected bromine.

The following organs were analyzed: blood, thyroid gland, cerebral hemispheres (mainly grey matter), hypophysis, peripheral nerves, gastric mucosa, and the liver. Weighed samples of the tissue to be investigated were ground in the cold in a mortar, and the proteins were precipitated by the addition of an equal volume of 10% trichloroacetic acid solution. The separated protein precipitates, also containing lipids, were washed 4-5 times with trichloroacetic acid with the addition of a small amount of inactive NaBr (to flush out the adsorbed Br^{82}), and dissolved in weak alkali. The protein solutions were transferred quantitatively to brass disks and dried to constant weight, after which their activity was determined. The trichloroacetic filtrate and washings were pooled and evaporated down to minimal volume, after which they were transferred to disks for final drying and determination of their activity.

Distribution of Br^{82} between the Protein and Protein-Free Fractions (in %) of Various Organs and Tissues of Animals
(Activity of 100 mg Tissue in Fissions per Minute is Given in Parentheses)

Animal	Duration of expt. (h)	Blood		Thyroid gland		Cereb. hemispheres		Hypophysis		Nerves		Gastric mucosa		Liver	
		protein	protein-free frac.	protein	protein-free frac.	protein	protein-free frac.	protein	protein-free frac.	protein	protein-free frac.	protein	protein-free frac.	protein	protein-free frac.
Rats	15	–	–	4 (136)	96 (3290)	–	–	–	–	–	–	–	–	–	–
	16	–	–	10.9 (182)	89.1 (1490)	–	–	–	–	–	–	–	–	–	–
	11	–	–	14 (570)	86 (3500)	–	–	–	–	–	–	–	–	–	–
	17	–	–	7.6 (154)	92.4 (1860)	–	–	–	–	–	–	–	–	–	–
	17	–	–	1.5 (27)	98.5 (1775)	–	–	–	–	–	–	–	–	–	–
	17	–	–	– (332)	–	–	–	–	–	0 (0)	100 (700)	–	–	–	–
Guinea pigs	16	–	–	9.3 (523)	90.7 (5114)	–	–	–	–	–	–	–	–	–	–
	18	0.4 (41)	99.6 (10050)	14.6 (3383)	85.4 (19790)	0 (0)	100 (6420)	0 (0)	100 (9450)	4.2 (77)	95.8 (1755)	0 (0)	100 (7200)	–	–
	18	–	–	20.2 (454)	79.8 (1790)	–	–	–	–	–	–	–	–	–	–
	16 h, 30 min	0 (0)	100 (4960)	5.9 (332)	94.1 (5280)	–	–	–	–	–	–	–	–	–	–
Guinea pigs	16 h, 30 min	–	–	6.4 (346)	93.6 (5020)	–	–	0 (0)	100 (2709)	–	–	–	–	–	–
Rabbits	16	1.3 (328)	98.7 (25000)	1.9 (545)	98.1 (28400)	0 (0)	100 (6000)	1.3 (132)	98.7 (8900)	0.2 (41)	99.8 (20180)	–	–	0.7 (59)	99.3 (789)
	21	0 (0)	100 (6080)	7.1 (368)	92.9 (4850)	–	–	0 (0)	100 (2360)	0 (0)	100 (3000)	0 (0)	100 (3200)	–	–
Dog	18	0 (0)	100 (3740)	23.6 (590)	76.4 (1910)	0 (0)	100 (682)	0 (0)	100 (1645)	0 (0)	100 (2420)	0 (0)	100 (2880)	–	–

EXPERIMENTAL RESULTS

The results showing the activity of the protein and protein-free fractions of the various organs and tissues, calculated per 100 mg weight of sample, are shown in the table.

It will be seen from the table that in 3 of 5 experiments the whole activity in the blood was found in the protein-free filtrate. In the remaining 2 experiments nearly the whole of the activity was found in the filtrate, and only traces of activity were associated with the protein precipitate.

In the thyroid gland of all the investigated animals most bromine was found in the protein-free filtrate. The amount of bromine combined with protein varied from 1.5 to 23.6%. These results show that bromine bears a specific relationship to the thyroid gland.

All the activity of the cerebral hemispheres and the gastric mucosa was found in the protein-free filtrate.

In the hypophysis in 4 of 5 experiments, and in the peripheral nerves in 3 of 5 experiments, the whole of the activity was found in the protein-free filtrate. In the remaining experiments only traces of radioactivity were found in the protein precipitate.

Since trichloroacetic acid is a comparatively harsh protein precipitating agent, we used others with a more gentle action, such as alcohol, acetone, ammonium sulfate, and mercuric acetate. In this series special attention was paid to the brain tissue and the hypophysis. However, in these tissues and with these precipitating agents, in no case was activity found in combination with proteins, whereas in the thyroid gland we invariably found some radioactivity in the protein fraction.

In order to discover the form in which bromine was present in the protein-free filtrate, the following investigations were conducted. In one series of experiments the protein-free filtrate of the brain tissue, containing all the Br^{82}, was dialyzed against tap water for 16-18 h. The undialyzable portion of the filtrate was then evaporated to dryness, dissolved in a small volume, and transferred quantitatively to disks for determination of their activity. The result of the determination was zero. This proved that all the bromine in the brain is present in a dialyzable form.

In another series of experiments, $AgNO_3$ solution was added to the protein-free filtrate with known activity, until precipitation was complete. Determination of the activity in the solution and in the precipitate showed that the latter contained the whole activity of the filtrate. Since only bromide ions (Br^-) give a precipitate with silver, this proved that all the bromine in the brain tissue was present in the ionic form.

Similar results were obtained for the hypophysis. No bromine combined with protein or in any undialyzable form was present in the hypophysis; all the bromine there was present in an ionic form and, consequently, the hypothesis that a bromine-containing hormone is present in the hypophysis was not substantiated.

The following point must be emphasized: The quantity of bromine in the hypophysis, determined by microchemical and radiometric methods, was always less than the quantity of bromine in the blood [5]. By taking this into consideration, and remembering that this gland is only very small, we must refute categorically the hypothesis that the hypophysis is a depot for bromine in the body.

SUMMARY

A study was made of the form of bromine existence in the blood, thyroid gland, brain hemispheres, hypophysis, peripheral nerves, gastric mucosa, and the liver in a number of laboratory animals. After the administration of sodium bromide (Br^{82}-labelled) fractionation of the animal organs and tissues was done, and then the activity of different biochemical fractions was determined. In the investigated organs and tissues bromine was in ionic form. The thyroid gland was an exception, where a part of bromine (from 1.5 to 23%) was protein-bound.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
