THE METABOLISM OF BROMINE.

-by-

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THESIS

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A C K N O W L E D G M E N T.

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INTRODUCTION

The element bromine was discovered by Balard in 1825 in Mediterranean water. In contrast with the other halogens, little is known concerning the distribution and physiological importance of this element.

Several theories have been advanced to explain the role of bromine in the living organism. Thus Bernhardt and Ueko(9) claim the possibility of an endocrine control of blood bromine. Zondek and Bier (119,120,121) and Pincussen (86,87,88) consider bromine as an important inorganic catalyser, in organic combination with protein, with an importance similar to iodine. On the other hand, Bürgi (11) explains the presence of bromine in the organism as purely dietary.

To obtain an understanding of the physiological function of bromine, if any, it is essential to know its normal distribution in both plant and animal tissues. The data available from the literature are both scanty and unreliable owing to technical difficulties encountered in bromine estimations. For this reason it was deemed advisable to study this phase of the problem in detail by using the modified Francis-Harvey-Yates procedure as previously outlined (cf.78).

A second method of approaching the problem was the estimation of the potential storage capacity for administered bromide of the various tissues, using rabbits as an experimental animal. It is reasonable to suppose that if an organ showed a specific storage capacity a relation to bromine metabolism could be surmised. This hypothesis is analogous with the retention capacity of the thyroid to administered iodine.

DISTRIBUTION OF BROMINE IN PLANT AND ANIMAL MATERIAL.

Marine Plants.

Algae.

Very little information can be obtained from the literature concerning earlier data on the bromine content of algae. Toller (99), analysing Nereocystis Lütkeana from Puget Sound, found in two analyses 0.11 and 0.19 % of bromine and 0.23 and 0.30 % of iodine respectively. The method used was not given. Wolff (112) gives several bromine analyses on the ash of European algae. These are, in percentages, Fucus vesiculosus, 0.62 ; F. servatus, 1.07; Halidrys siliquosa, 0.65; Laminaria digitata, 0.80; L. saccharina, 0.25. It is probable that much bromine was lost in the ashing of these plants.

Kylin (57), in a systematic study of the iodine content of a large number of algae, collected on the West coast of Sweden, carried out qualitative tests for bromine and found it present in most of the algae examined. Sauvageau (93), being unable to extract the bromine from algae with water before ashing, but readily after, concluded that most of the bromine is present in organic form.

Eel-grass.

Losana and Croce (63) found 0.061 % of bromine and 0.127 % of iodine present in Zostera marina collected off the northern coast of Africa, while Kylin (57) found no bromine in the same plant collected off the coast of Sweden.

Land Plants

Fungi.

The only data in the literature on the bromine content of fungi are those of Damiens and Blaignon (26). It seems of interest that the bromine content tends to be relatively high among the edible varieties, reaching a concentration of 3.6 mg. per 100 gm. dry material in Boletus scaber.

Flowering Plants.

Damiens and Blaignon (25,26) have shown that bromine is a normal constituent of the vegetable kingdom. For plants, with the exception of fruits, the amounts of bromine present vary between 0.17 and 2.02 mg. per 100 gm. dry material. The bromine content of fruits tends to be low, especially in the Rosaceae family, while muskmellon, watermellon and tomato on the contrary show a high bromine content (5.34 to 26.2 mg. per 100 gm. dry material).

Marine Animals (Invertebrates).

Phylum Coelenterata. Mendel (70) found no trace of bromine in Gorgonia acerosa. Cook (43) found none in the skeleton or coenenchyma of 10 species of Gorgonacia. The inability of these authors to demonstrate the presence of bromine is undoubtedly due to a faulty technique.

Quantitative observations have been made on corals by Mörner (74,75). The figures published are all given for the organic substance of the skeleton, "Gorgonin", freed as far as possible from other tissues and from inorganic material.

His figures may therefore be partially comparable with those given by the writer. Mörner carried out a large number of analyses on material collected at various parts of Europe and this continent. His results, given in a condensed form, are in percentages: (Gorgonaceae) Isidae, 0.74; Primnoidae, 2.94 - 3.76; Muriceidae, 1.18; Plexauridae, 0.96 - 4.20; Gorgonidae, 0.23 - 2.16; Gorgonellidae, 0.66 - 1.98; (Pennatulaceae) various species, 0.97 - 1.89; (Antipathidea) 2 species, bromine absent; 2 species, 0.38 and 1.53.

From Primnoa lepadifera Mörner was able to separate, by means of a baryta fractionation, a bromine-containing substance (76). This he subsequently identified as 3:5-dibromtyrosine.

Phylum Vermes. The only observations on annelids in the literature seem to be those of Mörner (74,75), who found that the tubes of Chaetopterus norwegicus and Hyalinaecia tubicola contained, after careful removal of calcium carbonate, 0.18 and 0.12 % of bromine, respectively.

Phylum Mollusca. No pertinent data for Mollusca were found in the literature, but it is interesting to note that Friedländer (43) has shown that the colored substance of Purpura and Murex is a dibromindigo.

Vertebrates.

Numerous observers claim to have shown the presence of bromine in marked amounts in the thyroid and pituitary glands. These will be dealt with in turn, and subsequently figures available for body fluids and the remaining body tissues will be considered.

Thyroid.

Damiens (22) in a single analysis could detect no bromine in a dog thyroid. The analyses of Bernhardt and Ueko (8,9), Lobat (62), and Serbescu and Buttu (94) indicated that appreciable amounts of bromine are present in dog, cattle and human thyroids. Tanino (97,98) and Jacobson (48) have considered a possible relation between the thyroid gland and bromine metabolism. They believe that the thyroid probably produces a bromine-containing substance, perhaps dibromthyroxine. Taxopeus (101) claims that the feeding of thyroid, thyroidectomy and the injection of posterior pituitary extracts into animals cause a change in blood bromine.

Pituitary.

Bernhardt and Ueko (8,9) were the first to claim that considerable quantities of bromine are present in the pituitary. Zondek and Bier (119,120,121), using an even less accurate method of analysis, claimed that the anterior lobe of the pituitary contains a considerable amount, but that the posterior contains only traces. Jacobson (48) makes similar claims.

From the results obtained by them, Zondek and Bier claimed that a bromine-containing endocrine principle is present in the anterior pituitary. They further claim to have isolated from the anterior pituitary a hydrolysable substance containing bromine which, when injected into dogs, produces signs of fatigue and asthenia. By analogy they supposed that the substance is tetrabrom thyroxine.

Such statements, if true, would obviously be important, and H. Zondek (118) has even gone so far as to suggest the presence of a bromine hormone in the pituitary.

These findings, however, have been contradicted by Serbescu and Buttu (94), and Dixon (28,29). Dixon finds that the amount of bromine normally present in the pituitary is of the same order as that of blood, and that the "Br/Cl" ratio in the pituitary of hogs is approximately the same as for the other tissues.

Blood.

The subject of bromine metabolism became especially interesting after Zondek (120) stated that blood bromine is lowered in manic-depressive states, and also in some organic cerebral diseases. These statements were supported by Sacristán and Peralta (92), Klimke and Holthaus (52), Kuranami (55,56) and others. However, Urechia and Retezeanu (104,105), Charvat and Hejda (20), and Yates (117) found that results in various pathological conditions show very little constancy. Dixon (28,29) finds that blood-bromine in manic-depressive psychotics, untreated with bromide or iodide for at least six months, exhibit the same type of variability in their blood bromine as normal subjects.

Concerning the distribution of bromine between the corpuscles and plasma, Guillaumin and Merejkowsky (44) found the ratio "corpuscle Br/plasma Br" higher than the corresponding ratio for chlorine, while Hastings, Harkins and Liu (47) obtained a smaller value. Hastings and Van Dyke (46), and Leipert (58) found that normally there is a slight retention of bromide in the corpuscles, in agreement

with the views of Böniger (12) on the permeability of bromide ion. The bromide distribution between cells and plasma is not a constant figure, but dependent on the CO_2 content of blood, similar to chloride (Hastings and Van Dyke (46), and Leipert (58)).

Guillaumin and Merejkowsky (44) stated that 63 to 88 % of the blood-bromine is in organic combination; Ewer (33) found 55 to 78 %; while Ueko (102) found only 20 % of blood-bromine in organic combination; (he states that it is not in combination with plasma proteins). On the other hand, Yates (117) states that all bromine in blood is in inorganic form. This latter work has been verified by Leipert (58) by means of deproteinization, dialysis and ultrafiltration, and this conclusion is almost certainly correct; the findings of Guillaumin and Merejkowsky, Ewer, and Ueko must be rejected.

Cerebrospinal Fluid.

The chlorine content of cerebrospinal fluid is always higher than that of blood plasma, in accordance with the Donnan equilibrium. Mishkin, Ritchie and Hastings (72) found that bromine in the cerebrospinal fluid is always lower than the corresponding concentration in blood plasma. After the administration of bromide, Walter (110) observed a similar distribution. He found the ratio of "plasma Br/ cerebrospinal fluid Br" normally to be 2.9 - 3.3. Leipert and Watslawek (60) have obtained similar results, although they point out that this rather indicates a difference of permeability to anions, and not to bromide in particular.

Gastric Juice.

Nencki and Schoumov-Simanovsky (77) were the first to show that when bromide is administered it replaces part of the chloride in the gastric juice. Quastel and Yates (91) observed a drop of 25 to 30 % of blood bromine following a meal. Leipert (58) has obtained similar results. The actual contents in his four analyses of gastric juice were 0.312 to 1.226 mg. %, each one higher than the corresponding blood bromine. Ueko (102) found the value to be 0.5 to 0.9 mg. per 100 c.c. in gastric juice from the "fasting" stomach.

Urine.

The excretion of bromine in urine in absence of specified bromine administration has been determined by a number of authors. Leipert (58) found this value to be 3 to 5 mg. per 24 hrs.; and Ueko (102), 1.0 - 2.5 mg. Both authors and also Hastings, Harkins and Liu (48) state that the ratio "bromine/chlorine" is always slightly lower in urine than in blood.

Tissues.

Damiens (22,23,24) followed by Bernhardt and Ueko (8,9), and Dixon (28,29) have determined the bromine content of a large number of animal tissues. The values obtained by Damiens are of the same order as those obtained by Dixon. The values obtained by Bernhardt and Ueko are somewhat higher. The results obtained by Justus (50) are certainly too high.

THE HALIDE DISTRIBUTION IN THE BODY IN CHRONIC BROMIDE INTOXICATION.

Nencki and Schoumov-Simanovsky (77) first showed the essential physiological action of bromide to be that of replacing the chloride in the organism. Determining the distribution of administered bromide in the various organs of the dog, Toxopeus (100) obtained results quite different from those of chloride by Wahlgren (109) and Fadtberg (82). While the normal chlorine distribution in the dog's organism is, skin 35 %, muscle 18 %, skeleton 18 %, and blood 12.4 %, up to 50 % of the administered chloride is stored in the skin. Administered bromide is, 37 % in muscle, 16 % in the skin and 9.3 % in blood. The bromide and chloride contents in single organs vary considerably; thus the ratio "Cl/Br" in the same animal and tissue may vary between 10 and 30 without causing any pharmacological effects. In Appelmann's experiments (3), the figure 40 % given by Ellinger and Kotake (32) as the maximum extent to which chloride may be replaced by bromide was frequently passed without apparent danger.

Oppenheimer (81) has shown that in a bromide treated dog the benzol extract of brain contains small amounts of bromine, while the lipides are affected to a much less extent by chloride. Later work (Mason (68), Leipert (58)) has not produced any evidence for the existence of organic bromine combinations in the body.

The quantitative application of the Gibbs-Donnan membrane equilibrium theory to blood and extracellular fluids by Van Slyke et al (96) led to the examination of the behavior of bromide (46) and the demonstration of its anomalous distribution between serum and corpuscles (30). The distribution ratios of

bromide between cells and serum. Br_{cells} : Br_{serum} (expressed in mM per kilo of serum and cell water) were occasionally found to be above unity, and generally well above the accepted average chloride distribution of 0.67. Most of these observations were made after the intravenous injection of bromide salts or shortly after taking a large dose of bromide by mouth, and only very few were made following prolonged bromide administration. Palmer and Clarke (83), on the other hand, state that if sufficient time is allowed for equilibrium to be established, there is no preferential uptake of bromide by the cells.

The distribution ratio of bromide between serum and cerebrospinal fluid, Br_{serum} : $Br_{c.s.f.}$, has been reported to be of the order of 1.5 to 2.0 (72) and 2.4 to 2.8 (65), as contrasted with the average for chloride of 0.89 (38), or the value of 0.95 obtained by applying the Gibbs-Donnan Law. Mason (68) attributes this variation to the supposition that cerebrospinal fluid is secreted, but not in equilibrium with serum.

Urinary excretion studies have yielded conflicting results. The theory advanced by von Wyss (114,115,116) that the kidney does not distinguish between bromide and chloride has been confirmed (39,40,41,73) and denied (83,47,69). Hastings, Harkins and Liu (47) and Leipert (58) find the ratio "Cl/Br" always slightly higher in urine than in serum, the average ratio in serum and urine being as 1:1.5. Leipert (58) states that only 60 - 70 % of the bromine that is theoretically possible from the plasma ratio appears in the urine.

THE PHARMACOLOGICAL ACTIONS OF BROMIDES.

The therapeutic use of bromides as sedatives, especially in cases of genuine epilepsy, has produced a variety of opinions as to the mode of this action. Thus Bernoulli (10) states that bromide ions, which take the place of chloride ions, alter the aggregation state of cell colloids, probably in the direction of greater swelling, thus producing a functional change, while Bancroft and Rutzler (5) believe that bromides peptize reversible agglomerated protein colloids of the nervous system. A physico-chemical explanation has been given by Gärtner (42) as being due to an increase in the permeability of membranes which results in a decrease in irritability.

Von der Velden (106) and von Wyss (116) ascribe the sedative action of bromides as being due to the dechlorinating action of bromides, while Ellinger and Kotake (32) and Januschka (49) have made attempts to show a sensitivity of the nervous system towards the narcotic action of bromide. Probably the most convincing evidence is due to Pavlov and his coworkers (84) who have shown that bromide has no depressant action on the central nervous system, but rather a specific reinforcing effect upon all its inhibitory activities.

A loss of chlorine from the body and a corresponding rise of bromine may produce symptoms of poisoning, generally referred to as "bromism". Several interesting reviews have appeared in the literature within recent years dealing with this condition (113,61).

THE DISTRIBUTION OF BROMINE IN INORGANIC MATTER.

At this stage it will be convenient to deal briefly with the present state of our knowledge regarding the distribution of bromine in inorganic matter. The data included, except where otherwise specified, are taken from Abegg (1) or Gmelin (43).

Bromine occurs in nature chiefly as bromides, and in solution as bromide ions. In the elementary composition of the earth (up to 10 km. depth) bromine occupies the 25th position (Vogt). The relative amount of bromine has been calculated as 1.10^{-3}%. The ratio of occurrence of the three halogens is Cl:Br:I = 250:1.0:0.1 (Vogt). Ackroyd (2) calculates the amount of bromine on land and in the sea as being 0.000583 and 0.01 - 0.015 % respectively. The solvent denudation for untold ages has eventually carried most of the bromine-salts to the sea, which is its great receptacle (Vernadsky).

Numerous analyses have been carried out on the bromine content of sea water. The only recent analyses show, in gm. per 100 c.c., for the Atlantic 0.0067 Br to 20.66 Cl (67), for the Adriatic 0.0064 Br to 18.38 Cl (111), and for the less saline Strait of Georgia, British Columbia, 0.0042 Br to 1.178 Cl at the surface, and 0.0056 Br to 1.559 Cl at a depth of 10 fathoms (17).

Bromine is present in varying amounts in mineral waters and salt beds (left by previous ocean waters). French mineral waters contain from traces to 8.2 gm. bromine per litre; German mineral waters contain from none to 1.880 gm. bromine per kg. Salt beds in Germany rich in Carnallite ($MgBr_2.KBr.6H_2O$) contain between 0.20 and 0.35 % of bromine; those in Solikamsk, Russia, contain 0.17 to 0.30 % of bromine (Efremov and Veselov-

skii (31). On this continent appreciable amounts of bromine are found in the salt beds of Michigan, West Virginia, Ohio and Pennsylvania. Various oil-bearing waters in Russia contain similar amounts (Maksimovich (64), Vinogradov (108)).

Bromine has been found as silver bromide in Chile, Mexico and France (Berthier), or as an iso-morphic mixture of silver chloride-bromide (embolite) in Chile, Mexico, Honduras and other localities. Occasionally it is found in the form of silver bromide-iodide.

The mother-liquors of Chile saltpeter contain bromide and also a small amount of bromate (Grineberg). According to Mitz, the bromate is formed from bromide by the action of microorganisms.

EXPERIMENTAL WORK.

PART I.

THE ESTIMATION OF BROMINE IN PLANT AND ANIMAL TISSUES.

As a result of the preliminary work (78) the modified Francis-Harvey incineration, combined with the Yates' oxidation technique, has been adopted as a standard procedure and thoroughly tested. It is outlined below.

The Modified Francis-Harvey-Yates Procedure.

(a) Ashing. Introduce the substance to be analysed into a 150 c.c. Ni crucible. Add 5 c.c. N KOH, 0.1 c.c. 20 % sucrose solution (to prevent formation of bromate) and 10 c.c. of distilled water. Mix well. Evaporate to dryness on a water-bath, heat at 150°-160°C. for 1 hour, and ignite in a muffle furnace for 1 hour at 475°-485°. Cool. Add 20 c.c. water, break up the carbonised mass with a glass rod, digest on the water-bath for 2-3 minutes, and decant through a filter paper (9 cm. Whatman, previously washed with hot distilled water) into a second, clean Ni crucible. Repeat the extraction, and mix the extracts together. Place the filter paper in the first crucible, moisten with water and 1 c.c. N KOH, evaporate to dryness, heat at 150°-160° for 15 minutes, and at 475°-485° for 1 hour. Cool; extract residue as before. Evaporate the combined extracts to dryness, heat at the two specified temperatures for 15 and 10 minutes respectively, cool, dissolve in 10 c.c. water, evaporate, and repeat the short ignition. To remove the last traces of organic matter, dissolve in 5 c.c. water, add a small crystal of KNO_3 (about 1.5 mg.), evaporate, and repeat the ignition.

Treat the residue with a little water and filter (7 cm. Whatman, treated as before) into a third crucible. Wash the crucible and filter with successive portions of water to make the filtrate up to 30 c.c. Evaporate to dryness and ignite at the two temperatures for 15 and 5 minutes respectively. (If the material has to be kept overnight, the third crucible should be of platinum.)

(b) Oxidation. Dissolve the contents of the crucible in 3.0 c.c. distilled water, accurately measured, and transfer to a 100 c.c. Erlenmeyer flask. Wash the crucible with two further 2.0 c.c. portions of water. The flask is fitted with a two-holed rubber stopper (rubber does not seem to affect the results of this particular procedure). Incline the flask at an angle of 60^{0} to ensure maximum aeration. The stopper carries two tubes, one slightly drawn out and reaching the bottom of the inclined flask, the other bent to 60^{0} and connected to a small bubbler containing 1 c.c. 10% KI and 4 drops of 1% starch. Run down the inside of the flask 2.5 c.c. of concentrated H_2SO_4, with constant shaking and cooling under the tap. This must be done very slowly and should take at least 10 minutes. Then run in 4 c.c. of chromic acid-sulphuric acid mixture (20 g. chromic acid, 40 c.c. concentrated H_2SO_4, sp.gr.1.84, and 120 c.c. distilled water). Insert the stopper and aerate 1 hour. Change the receiver and aerate a further 2 hours. Titrate the liberated iodine against N/500 thiosulphate from a micro-burette.

Blanks must be determined from time to time, and the figures found subtracted from actual determinations. Usually a perfect blank is obtained.

A set of checks with known quantities of bromine (dibrom-tyrosine, or potassium bromide, or both together) have been run about once a week throughout the period of this work, with uniformly consistent results, typified in Table I. It will be seen that the results tend to be too low by a negligibly small amount.

PART II.

THE DISTRIBUTION OF BROMINE IN PLANT AND ANIMAL (INCLUDING HUMAN) MATERIAL.

Experimental Results.

A systematic study of the normal bromine content of as great a variety of material as was easily available has been made. A portion of the material used was collected in the summer of 1913 and 1914 by Dr. A.T.Cameron, mostly at the Pacific Coast Station of the Dominion Biological Board (at Departure Bay, British Columbia). These samples have all been kept in stoppered bottles, but, to remove any water that might have been taken up in the long period of storage, each sample was heated in a 100^{0} oven to constant weight. The approximate localities from which these samples were obtained are as follows:

(a) At the Biological Station, Departure Bay, B.C., or at points within half a mile of it.

(b) Near Snake Island, 2 miles east of (a).

(c) From the sand flats off Protection Island, 2 miles south-east of (a).

(d) In False Narrows, about 8 miles south-east of (a).

(e) At Nanoose, 10 miles north-east of (a).

(f) At North West Bay, 20 miles south-east of (a).

(g) At Belle Chain, 50 miles south-east of (a).

(h) South of Mudge Island, 2 miles south of (d).

(i) Off Suquash, north of Vancouver Island, B.C.

(j) From Alaskan waters.

(k) From the Canadian Atlantic Coast.

(m) From the Marine Biological Station, Plymouth, England.

My sincere thanks are due to Dr. W.F. Geddes of the Dominion Grain Commissioners' Research Laboratory in Winnipeg, who kindly prepared especially for these analyses 23 samples of wheat, consisting of No.1 and No.2 Manitoba Hard. These are pooled samples of some 46,000 individual samples from the 1933 and 1934 crops, made up according to 23 different protein-content areas of the three western prairie provinces, as follows:

(n) Alberta

(o) Saskatchewan

(p) Manitoba.

The remainder of the plant material was obtained at the following points:

(q) Winnipeg, within a radius of 40 miles.

(r) Carnduff, Saskatchewan.

(s) Delta Manor, Ladner, British Columbia (about 4 miles from the ocean-shore.

(t) California.

With the exception of the cereal grains, all the new material was dried in a 100° oven to constant weight. The cereal grains were analysed in the fresh condition.

The tissues from rats (pooled samples from five females and five males), rabbits (pooled samples from one female and one male), and dog (female collie), and the other material obtained in the laboratory, were dried in an oven at 100°. The rabbit thyroid, pituitary and ovary materials are pooled samples from 24 rabbits used in Friedman tests.

The thyroglobulin and thyroid nucleoprotein preparations were obtained from Dr. Cameron. The first-mentioned was prepared by the Oswald procedure, purified by repeated treatment with 1 % NaCl and $(NH_4)_2SO_4$, dialysed against distilled water, and precipitated with alcohol.

Of the endocrine products reported on in Table VI, the Winnipeg material was collected from a local slaughterhouse (Burns and Co.) to whom I am grateful for the facilities extended to me. The hog thyroids and the American samples of pituitary glands were kindly placed at our disposal by Dr. Frederic Fenger of the Armour Organotherapeutic Laboratories for the purpose of this work. The sheep thyroid preparation was obtained from the same source. The samples labelled "commercial" were purchased in the usual way.

On account of the possible interrelationship of halogens, it seemed desirable to include as many iodine analyses as possible. All the iodine values in Tables II and IV are taken from Dr. Cameron's paper (13 to 16). A certain number of iodine analyses have been carried out by the present writer using Kendall's method (51).

The human material was obtained through the courtesy of Drs. D.C.Aikenhead, Sara Meltzer and O.C.Trainor. Two of these persons were accidentally killed. These, a boy of 14, case 1, and a woman of 50, case 2, were apparently in normal health at the time of death. Of the others, a man of 70 and a man of 71, cases 3 and 4 respectively, died from heart failure (cardiac myocarditis). A woman of 50, case 5, died from cerebral haemorrhage. No definite lesions were found in any of the tissues examined in the last three cases.

All analyses of human tissues, with but three exceptions, were carried out in duplicate, and similar good agreement was obtained to that shown in the previous analyses. Only the mean results, therefore, are given.

In a recent paper, Ueko (102) claimed that there is a definite relationship between the bromine and chlorine content in mammalian tissues, while Leipert (58) has also claimed that such a definite ratio exists in blood, urine and gastric juice. The author has accordingly analysed a large number of tissues for total halogen, using Van Slyke's method (95), and the results (means of duplicate determinations) are also given in Table IX, expressed in terms of chlorine.

In the last column of Table IX are given the equi-atomic distribution ratios of bromine and chlorine in terms of chlorine. The necessary corrections for iodine have not been made, except for the thyroid material. Since the iodine contents of other tissues are extremely small, the values taken for chlorine are sufficiently accurate.

The results obtained are given in Tables II to IX. Certain isolated determinations will be mentioned later in the discussion.

Comparison of results with those of earlier investigators.

Since undoubtedly many of the analyses recorded in the literature are based upon inaccurate methods, such results must be accepted with reservations, and it seems desirable to attempt to evaluate the procedures adopted by other investigators through experience gained in establishing the accuracy of the method adopted. The following appears to be legitimate appraisal of the data quoted in this work from earlier investigators.

Justus(50) fused to a dry ash, liberated bromine with HNO_2 and took it up with $CHCl_3$. His results are extremely high, due to the uncontrolled use of HNO_2.

Lobat (62) fused to a dry ash, liberated bromine, and measured the colour developed with fluorescein. His results were usually negative, and his method undoubtedly was not sufficiently delicate. The same criticism applies to the methods of Baubigny (6), and Serbescu and Buttu (94).

Walter's method (110) applies only to protein-free filtrates from blood. $AuCl_3$ is added and the colour developed is matched against a standard. Comparable but not absolute accuracy is obtained with this method (cf.Malamud, et al (66)).

Damiens (22,23) used fusion with alkali and the development of a specific colour with fuchsin, first described by Denigès and Chelle (27). His method is probably moderately accurate (cf.Olszyeka (80)).

Bernhardt and Ueko (8) fused with alkali, and employed a modification of Damiens' colorimetric procedure. According to Olszyeka (80) the method is inaccurate and the results it gives are too high.

Roman (89) used fusion with alkali, liberation of bromine by HNO_3-H_2O_2, extraction with $CHCl_3$, and titration with thiosulphate. His method has been adversely criticised by Fleischbacker and Scheiderer (35,36) and by Hahn (45); undoubtedly many results obtained by it are too high.

Behr, Palmer and Clarke (7) used fusion with alkali, and liberated free bromine by H_3PO_4-$KMnO_4$ mixture, extracted it with CCl_4, and titrated with thiosulphate. The method has not yet been tested by other investigators, but is possibly open to the same type of criticism as applies to that of Roman.

Guillaumin and Merejkowsky (44) oxidized in acid-permanganate solution, and then followed a method with fuchsin based upon that of Damiens. The accuracy of their results is probably similar to his.

Francis and Harvey's method (37) has been found inaccurate by the present writer (cf.78).

Dixon (28) fuses with alkali, and converts to bromate, which is estimated iodometrically. This type of procedure, carried out under carefully controlled conditions, has been found accurate by Heulen (71) and by Kolthoff (53).

Ueko (102) has recently modified the method of Bernhardt and Ueko (8). This new method has not as yet been checked by other writers, and may be open to the same criticism as the previous one.

Leipert and Watslawek (59) oxidize the material by a silver-sulphate-chromic acid-sulphuric acid mixture. Iodine is retained as iodate; chlorine and bromine are aspirated into NaOH solution, the bromine is changed to bromate with NaClO and estimated iodometrically. The method has been found to give good results (78).

The results of previous authors, in so far as they can be related to dried or fresh plant and animal tissues, are given in Tables X (plants), and XI and XII (animal tissues). The figures in Table X refer to percentage of bromine in dried material, and are all from Damiens' papers; they are determined by his method, which probably leads to fairly accurate results. Those in Tables XI and XII are referred to fresh material except where otherwise stated. In these tables, the author's reference is given in parentheses after the percentage figure (or at the heads of columns in Tables X and XII); when the method used is considered to give unreliable results, these also are placed within parentheses. My own results are inserted for comparison ("W" in parentheses).

Pincussen (86,87,88), Zondek and Bier (119,120,121), Jacobson (48), Ewer (33), Kuranami (55,56), Urechia and Retezeanu (104,105) and Tanino (97,98) used Roman's method (89); Kriwskii (54) used Bernhardt and Ueko's method (8); Pillet (85) used Baubigny's method (6); Chelle (21) used Denigès and Chelle's method (27).

With the exception of blood, few analyses for human tissues based upon sound methods are available in the literature. These are contrasted in Table XIII with those of the present writer made on the same tissues. Ueko's figures are included since his new method has not as yet been checked. It will be seen that the figures are of the same order, but show wide variations which are not improbably due in part to varying bromine intake (in diet and perhaps in medication, in the cases studied).

Among the figures in the literature which appear to be too low on account of employment of unreliable methods are those of Pribram (90) (no bromine present in the brain, liver, pancreas or thyroid), Lobat (62) (no bromine in liver, heart, spleen, kidney, blood serum, 0.015-0.02 mg. % in the cerebrum, and 0.07-3.0 mg. % in the thyroid), and Serbescu and Battu (94) (no bromine in the liver, pancreas, heart, spleen, kidney, adrenal, pituitary, lung, muscle, and 0.415-3.15 mg.% in the thyroid). On the other hand Justus (50) analysed 12 organs and found bromine constantly present, the results varying from 14 to 122 mg. %; his method undoubtedly gave too high results.

Bernhardt and Ueko (8,9) have published a large series of analyses, but, as Olszycka (62) has shown, these results are also inaccurate and cannot therefore be considered for comparison.

DISCUSSION OF RESULTS.

The contrasted results in Tables X to XIII, on the whole, show differences to be expected from the criticism of the methods. The most extensive series, that of Damiens, tends to be somewhat lower than my own especially for some of his plant material, presumably from French sources, but is in good general agreement. The figures of Bernhardt and Voko are slightly but distinctly higher; those of Justus are obviously much too high and those of Lobat much too low.

Marine Plants.

Algae.

Of the Brown and Red algae examined by the writer, all contained appreciable amounts of bromine. The highest amount was found in the frond of Nereocystis lütkeana. With the exception of the frond of Macrocystis pyrifera, the bromine content was always higher than that of iodine; there seems to be no relation between the two.

The number of analyses carried out is insufficient to determine the degree to which environment and selective affinity respectively determine the bromine content of these plants, but comparison with the figures for land plants makes it obvious that environment plays a very important role.

Land Plants.

Flowering Plants.

The data for plants given in Tables III and X are sufficiently complete to show that bromine is present in a measurable amount in the great majority of flowering plants. The amount of bromine present in the environment is undoubtedly a determining factor of the amount present in the

plant. This is well illustrated in the case of Brassica oleracea (cabbage) grown in the summer of 1935 - the Manitoba material has a bromine content of 0.0002 %, while the British Columbia material has a content of 0.0025 %. In general, most of the British Columbia material has a higher bromine content than that of Manitoba, in agreement with the proximity to the ocean of the source of this material. The results also show that closely related species have markedly different selective affinities for the element. To produce conclusive evidence of this, however, it would be necessary to grow plants under the same conditions.

The tables show definitely that different parts of the same plant have varying affinities for bromine. The green parts invariably have a higher bromine content than the roots of the same plant (e.g. Zea mais, Triticum vulgare, Brassica rapa, Trifolium pratense, Daucus carota, Pastinaca sativa and Solanum tuberosum). In the case of Zea mais and Triticum vulgare, where the fruits as well as other parts of the plants were analysed, the fruit invariably contained the least amount of bromine. If this rule is generally applicable, it explains the low results for all cereal grains. In the two samples of Triticum durum grown in the same soil in 1933 and 1935, the 1935 sample has a bromine content of 0.0003 %, that of 1933 a content of 0.0001 %, suggesting a climatic factor.

Where comparisons are possible between my figures and those of Damiens and Blaignon (25,26), the former tend to be higher. The two species of melon examined by Damiens and Blaignon are unusually rich in bromine (0.00945 and 0.0262 %). I have had no opportunity to analyse similar material.

Marine Animals (Invertebrates).

Phylum Porifera. The four species of sponges examined were all non-calcarious single specimens. I have found no reference in the literature as to the bromine content of Porifera.

The bromine content of the Pacific sponges examined is somewhat of the same order as that of iodine.

Phylum Coelenterata. The results obtained for the Pacific Jelly-fishes examined are remarkable for the fact that quite appreciable amounts of bromine are present, while iodine is present in negligible amount. For three of these coelenterates the water content had been determined (15), so that it is possible to demonstrate what proportion of bromine may be due directly to sea-water (cf. Table XIV). The sea-water from which these animals were taken contains about 4 mg. of bromine per 100 c.c. (17).

Table XIV shows that only in the case of the sea-anemone Metridium marginatum can all the bromine possibly be so accounted for. The table also stresses a different affinity for bromine of the cells of these three species of Hydrozoa.

The Verticillate fan coral from Alaskan waters gave the highest value obtained for any material so far examined, 0.354 % for the whole animal, and 0.876 % for the skeleton referred to as dry material.

Phylum Vermes, sub-phylum Annulata, class Chaetopoda, order Polychaeta. The annelid worm named by Dr. Cameron Sabella columbiana (15) was later identified as Eudistylia gigantea (79).

Dr. Cameron (15) makes the following statement as to the nature of the worm-tubes examined: " The Diopatra worm-tubes consist of an upper part, 4 to 6 inches in length, covered with shells and small Algae, and a lower part, up to 18 inches in length, of parchment-like consistency, consisting of concentric layers, the inner being translucent and usually perfect, the outer more or less damaged. The lower tube is secreted by the glands of Tori, the leathery upper tube in part is lip secretion. The tubes taken for examination were separated from adhering material (shells, Algae) and sand as far as possible, resolved into layers and air-dried. The Chaetopterus tubes had a similar structure to those of Diopatra. --------- The Sabella (Eudistylia) and Hispira tubes were tough, and horny in appearance, and consisted of numerous layers of translucent material. --------- The Phoronis tubes were of thin hyaline material. "

Only one sample of worm-tissue has been examined. This contained 0.0195 % of bromine. All the worm-tubes examined contained bromine, the limits observed being 0.0019 and 0.319 %. During the analyses it was noticed that most of the material contained varying amounts of sand. Since no silica analyses have been made on this material, the necessary correction is not known.

Phylum Mollusca. The dermis of the foot of the sea-whelk Schizotherus nuttalli is stated to be a secretion of the subdermis. In the two samples analysed the figures (in each case for one or two specimens only) indicate that the bromine content is of the same order as that of iodine.

The byssus of Mytilus is an adhesive secretion, and the opercula of Polynices a protective secretion. The bromine content of these is considerably higher than that of iodine.

Phylum Chordata, subphylum Tunicata. Only the tests of a few ascidians were examined. Bromine was an invariable constituent, the limits observed were 0.053 to 0.228 %, there being noted variations in different species.

The literature contains nothing on the bromine content of tunicates.

Vertebrates.

Bromine in Blood.

I have carried out analyses on two separate samples of beef blood obtained at a local abattoir. The amounts of bromine found present were 0.00071 and 0.00133 gm. per 100 c.c. A portion of the second sample was centrifuged (at 2500 revolutions per minute), and the bromine content of the plasma determined (52 % plasma and 48 % corpuscles); 100 c.c. of plasma were found to contain 0.00173 gm. of bromine, and 100 c.c. of corpuscles 0.00090 gm. (calculated).

Bromine in the Pituitary.

In Tables V and VI data are given of a few analyses carried out on rabbit, dog and beef pituitaries, and two pituitary preparations. The results obtained are in agreement with those of Dixon (28,29). The findings of Zondek and Bier (119,120,121) and others, that the pituitary plays an important role in the physiological function of bromine are almost certainly based on insufficient evidence.

Bromine in the Thyroid.

I have carried out a number of analyses of thyroid tissues of different animals, and of commercial thyroid preparations. Most of the thyroid material contains appreciable amounts of bromine; the limits observed are from a trace to 0.105 %. In all cases where the iodine content was also determined, it was considerably higher than that of bromine. The thyroid material from marine fishes has in most cases a higher bromine content than that of land mammals. This can probably be ascribed to diet.

Data are given in Table VII for several thyreoglobulin preparations and one thyroid nucleoprotein preparation; all these were prepared by Dr. Cameron in 1926. In the case of thyroglobulin, the iodine content is decidedly high, while that of bromine appears to be of the same order as that of desiccated whole thyroid tissue. The thyroid nucleoprotein preparation contained none, or only a trace of bromine. To obtain some further clue to a possible function of bromine in the thyroglobulin molecule, three lots of thyroid glands were treated with 1 % NaCl solution and bromine determined in the residue. The results show no constancy in the proportion of bromine extracted, nor in the ratio of iodine extracted to bromine extracted; relatively more iodine than bromine was extracted.

A further experiment was carried out on beef thyroid. It was found, expressing the results in terms of 100 gm. of fresh material, that of the total bromine present (1.8 mg.), 1.7 mg. were extracted by 0.1 M sodium acetate, and 0.1 mg. was found in the residue. From the extract 2.22 gm. of

impure thyroglobulin was obtained containing only 0.05 mg. bromine, so that the remainder in the sodium acetate extract (1.65 mg.) was not present in thyroglobulin. Calculated to dry weights, the bromine percentages in thyroid and impure thyroglobulin were 0.0078 and 0.0022 respectively. These experiments suggest that bromine is mainly associated with the non-thyroglobulin material of the thyroid.

Bromine in Tissues.

In the analyses on the various tissues of the rat, rabbit and dog reported, considerable variation in their bromine contents is noticeable. All the tissues analysed contained bromine, ranging from a trace to 0.0106 % (dry material). The values obtained are of the same order as those obtained by Damiens (22,23,24) and Dixon (28,29).

In my experiments the diets of the rats (bread, milk and vegetables), the rabbits (hay and vegetables) and the dog (dog biscuit and water for two weeks) all contained traces of bromine (see Table VIII). Damiens and Blaignon (26), and Dixon (29) have shown that common salt, invariably a constituent of the diet, usually contains a small amount of bromine. This indicates the difficulties encountered in endeavouring to interpret results for the normal bromine content of tissues. Variations found in a normal series of analyses of animal tissues are undoubtedly very largely due to differences in bromine intake.

Human Tissues.

Bromine in blood.

In addition to the figures recorded in Table XIII, the following apparently reliable values (in mg. %) have been published for individuals presumed normal, and not known to have been given bromine compounds therapeutically, Quastel and Yates (3 cases), 0.83-1.46 (91); Guillaumin and Merejkowsky (6 cases), 0.74-1.60 (44); Leipert (10 cases), 0.16-0.4 (58); and Ueko (100 cases), 0.15-0.35 (102).

The results reported in this work for normal blood are 0.62 to 1.01 mg.% (3 cases). The variation in bromine content is probably due mainly to differences in bromine intake.

Bromine in urine.

Several bromine determinations were made by the present writer on the urine of normal individuals (morning samples taken before breakfast). The results obtained were 0.00113, 0.00181 and 0.00185 gm. per 100 c.c. Though few, these results indicate that the amount present is not constant; presumably diet and concentration are the determining factors.

Bromine in the pituitary.

The assumption that the pituitary plays an important role in bromine metabolism, as set out by a number of authors, has not received any confirmation in the more accurate work of Dixon (28,29). The results obtained by the writer are in full agreement with those of Dixon.

Bromine in the thyroid.

Results previously mentioned appeared to indicate that the thyroid alone stored amounts of bromine definitely greater than could be ascribed to blood circulating through the different tissues. Results for human material also appear to suggest that this is the case. In addition the writer analysed certain thyroid material obtained from the operating theatre at partial thyroidectomy. The results obtained from bromine, iodine and total halogen are shown in Table XV in mg. %. All the operative cases had been treated with Lugol's solution prior to operation.

The results in Table XV show too great variations in the ratios of the three halogens to permit any conclusions beyond the fact that in the thyroid the ratio of bromine to chlorine is greater than in other tissue. This perhaps supports the view that bromine is concerned with thyroid metabolism, although the author could find no evidence that bromine is associated with the functioning compound of the thyroid.

The Relation of Chlorine, Bromine and Iodine in Plant and Animal Tissue.

No relationship appears to exist between the bromine and iodine contents of marine algae and marine animals.

Earlier figures for the "Br/Cl" ratio for human and other mammalian tissues (weight-ratios), can be summarised as follows ($all x 10^3$).

	Ueko (102)	Leipert (58)	Damiens (22,23,24)	Neufeld
Organs (not including thyroid)	1.5-8.0	-	0.21-4.53	1.19-5.86
Gastric juice	1.7-5.5	1.54-3.98	-	-
Blood	0.5-1.4	0.62-1.42	1.32-1.55	2.34-4.34
Urine	0.3-0.6	0.41-0.57	0.39-1.85	-

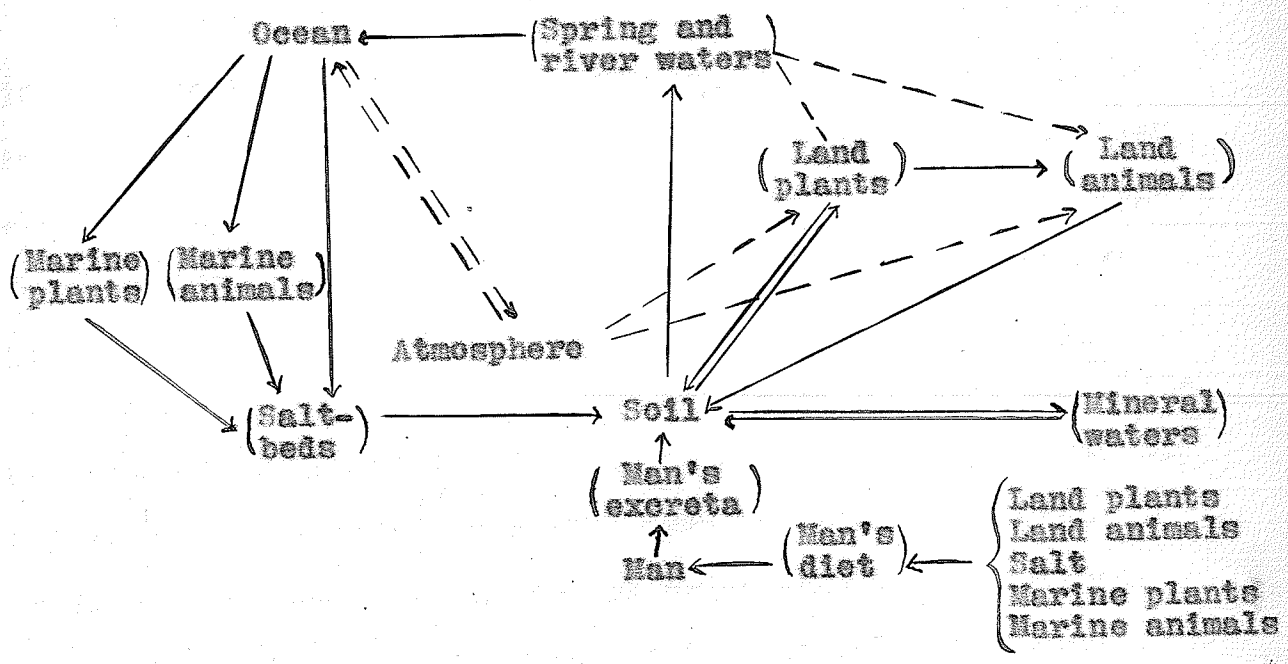
These figures, and the detailed figures in Table IX do not show sufficient constancy to suggest that any physiological ratio exists. The figures in Table IX suggest that the ratio in the thyroid is relatively much greater than in other tissues, but these are only based on two analyses, while Ueko (102) did not, in his analyses, find such high ratios. This finding, therefore, cannot be stressed.

Baldauf and Pincussen (4) find that the ratio Br/I in normal and various pathological cases varies from 97 to 105 in the blood. Tanino (97,98) finds that the ratio Br/I is higher in thyroid glands with a high colloid content than in those poor in colloid. My findings on bromine distribution in the thyroid scarcely support this view.

The Bromine Cycle in Nature.

The presence of bromine in marine plants and animals, land plants and animals, ocean-waters, mineral waters, and in the salt-beds left by previous ocean-waters, has been given. From this it can be concluded that bromine is present in most soils, but to a varying degree. Since the large concentration of bromine in ocean-waters is due to a constant denudation by the solvent action of waters, it must also be present (to a very small extent) in spring and river waters.

Fellenberg (34) has shown that soil and sea-water will give up iodine to the atmosphere. Since the concentration of bromine in sea-water is very much greater than that of iodine, it can be surmised that bromine is also present in the atmosphere. Taking all these data into consideration, the following schematic arrangement of the bromine cycle in nature seems justified. In it the dotted lines represent possible but unproved movements.



PART III.

THE BROMINE CONTENT OF DIFFERENT TISSUES OF THE RABBIT FOLLOWING THE ADMINISTRATION OF BROMIDES.

To clarify the relation of bromine to human physiology and therapy one has to understand its distribution in the organism, its excretion and chiefly its actions - such as its indifference to certain organs. With this in view, the following experiment was carried out.

Two male rabbits were given by subcutaneous injection 10.8 mg. of bromide (as sodium bromide) per kilo body weight twice daily for 10 days. The animals were kept in metabolism chambers and the rate of elimination of bromine in urine and faeces determined. The results for bromine elimination are given in Table XVI.

Rabbit 1 was killed the day following the last injection, while rabbit 2 was killed four days after the last injection. All tissues were dried in a 100° oven to constant weight, analyses being carried out on the dried material. Wherever possible analyses were carried out in duplicate; the mean results are given. The distribution of bromine in the various tissues are given in Table XVII.

In the last column of Table XVII is given the normal chlorine content of the different organs of the dog taken from the paper of Cameron and Walton (16).

Discussion of Results.

Rabbit 1 received during the 10 day period 538.9 mg. of bromide, while the excretion amounted to 134.2 mg. (24.9 %). Rabbit 2 received 463.6 mg. of bromide, and by the end of the 14th day had excreted 223.9 mg. (48.4 %). These results indicate a remarkably slow excretion, in agreement with Leipert (58), Hastings and van Dyke (46) and others.

The relative concentrations of the different organs in each rabbit are similar. There is no evidence to indicate any specialized tendency for bromine retention by any particular organ.

The general distribution of the administered bromide is blood > skin > muscle > bone. This is not in agreement with the results obtained by Toxopeus (100). As a whole the figures rather show a distinct similarity to the normal distribution of chloride in the organism.

The results obtained do not support the view held by Carnot and Coirre (19) and Vilém (107) that the largest amount of administered bromine is found in the brain.

It seems reasonable to conclude that the actual figures found for any tissue are largely dependent on the vascularity of such tissue and the amount of blood present within it.

To determine the amount of bromine present in organic combination in thyroid, if any, a weighed amount of thyroid sample was extracted with boiling water, filtered by suction, and the residue washed with boiling water. The collected filtrates were cooled, made up to volume, and the bromine content determined by analysis. The results obtained are

for rabbit 1, 43.29 mg. % and for rabbit 2, 20.90 mg. %. These results agree with the original figures. It is evident therefore, that all the bromine in the thyroid is present in inorganic form. Similarly it was shown that all the bromine present in blood is in inorganic form.

PART IV.

AN ATTEMPT TO ISOLATE BROMINE CONTAINING COMPOUNDS FROM EUDISTYLIA GIGANTEA.

Up to the present time, only two simple bromine compounds have been prepared from living tissues, dibromtyrosine from corals by Mörner (76) and dibromindigo from molusca by Friedlander (43).

In attempting to isolate bromine-containing substance from the products of hydrolysis it is advisable to avail oneself of material relatively rich in bromine (and at the same time containing none or only traces of iodine). The only material available in sufficient amounts was the worm-tubes of Eudistylia giganten, containing 0.152% Br and 0.975% I.

A number of attempts were made to concentrate the bromine compound (or compounds) from the hydrolysates of this material. A brief account of two such attempts is given.

The dried material (100 gm. containing 152 mg. Br and 975 mg.I) was boiled under a reflux condenser for 5 hours with 800 c.c. of 20 % solution of crystalline barium hydroxide; after cooling the solution was filtered, taken down to about 100 c.c. on the water-bath, cooled, and filtered a second time. The precipitates, which consisted mostly of undissolved barium hydroxide, were found to contain only traces of bromine; the final filtrate contained 140 mg. Br and 930 mg. I. To the filtrate was added five volumes of 95 % alcohol and this allowed to stand at room temperature for 48 hours. It was then filtered, and the precipitate washed with 95 % alcohol and dried. Analyses showed that this brownish-red residue

contained 116 mg. Br and 842 mg. I. This was taken up with water at about 40 to 50°, and to remove all barium present sulphuric acid was added. The filtrate from this was treated with a 20% phosphotungstic acid solution and filtered after one hour; the excess phosphotungstic acid was removed from the precipitate with excess barium hydroxide and the excess barium removed with sulphuric acid. The filtrate was made slightly alkaline with ammonium hydroxide, reduced in volume under diminished pressure and while still warm diluted with three volumes of 95 % alcohol. This was allowed to stand one day, filtered, and the alcohol from the filtrate removed under diminished pressure. The final solution, 20 c.c. of a brownish-yellow colour, contained 22 mg. Br and 220 mg. I.

Attempts to obtain crystals from the resulting solution failed. It gave a distinct colour reaction with nitrous acid and ammonia, indicating the presence of either diiodo-tyrosine or thyroxine.

Two hundred and fifty grams of material (containing 380 mg. Br and 2437 mg.I) was boiled under a reflux condenser with 500 gm. of barium hydroxide and 2000 c.c. water, allowed to cool and filtered. The filtrate was brought to pH 5 by the addition of 50 % sulphuric acid. The precipitate, containing 38 mg. Br and 192 mg. I, was filtered off; the filtrate and washings contained 339 mg. Br and 2240 mg. I. The filtrate was treated with basic lead acetate until no further immediate precipitation occurred; after standing for 24 hours the lead salts were filtered off and suspended in water; the mixture was heated to boiling and 50 % sulphuric

acid added until the reaction remained acid to Congo red. Lead sulphate was removed by filtration and the filtrate freed from sulphuric acid by addition of a slight excess of barium hydroxide. The alkaline filtrate contained 189 mg. Br and 1360 mg. I. This was treated with carbon dioxide and the barium carbonate removed by filtration; the filtrate and washings contained 180 mg. Br and 1200 mg. I. The filtrate was concentrated under diminished pressure to 200 c.c. This was extracted by shaking out seven times with butyl alcohol (previously purified by agitation with saturated sodium bisulphite followed by distillation) at a temperature of about 70°; the combined butyl alcohol extracts were evaporated to dryness under diminished pressure and the residue dissolved in 400 c.c. of water; the resulting solution contained 149 mg. Br and 1030 mg. I. The solution was brought to the boil and treated with uranium acetate solution in slight excess; the precipitate was filtered off and the filtrate freed from uranium with ammonia and concentrated to 295 c.c. under diminished pressure; it now contained 105 mg. Br and 900 mg. I. Basic lead acetate solution was added to complete precipitation, and after standing overnight the precipitate was collected and decomposed by saturating its suspension in hot water with hydrogen sulphide. The lead sulphide was boiled out with much hot water, and the filtrate and washings concentrated to a small volume under diminished pressure. The solution was neutralised to litmus with ammonia and further concentrated in a vacuum desiccator over sulphuric acid. No crystals were obtained. The brownish

residue obtained by drying gave a distinct colour reaction with nitrous acid and ammonia, indicating the presence of diiodotyrosine. Analyses showed the dried powder to contain 5.12% Br and 50.61% I.

Various procedures were tried to separate the bromine and iodine fractions in the residue. Some success was obtained by the following method.

An aliquot of the residue was covered with concentrated hydrochloric acid and boiled under a reflux condenser for three hours; this was cooled and an excess of barium hydroxide added; heated on a water-bath for three hours, cooled and filtered. To the filtrate were added five volumes of 95% alcohol and this was allowed to stand for 24 hours. A brownish precipitate settled out containing 46.01% Br and no iodine; the nitrogen content, as determined by the micro-Kjeldahl, was 4.02%. It gave a negative reaction with nitrous acid and ammonia.

The residue, from which no crystals could be obtained, gave a negative Millon's reaction. All the bromine present went into solution on heating in water with zinc dust. The residue obtained contained no bromine and gave a positive Millon's reaction; it developed a blue colour with phospho-molybdotungstic acid (phenol) reagent. This indicates the development of tyrosine.

The residual amount was so small, however, that since all endeavours to obtain crystals from it failed, an attempt to isolate the substance in pure condition was abandoned. It became evident that larger initial amounts of the material were necessary.

It seems justifiable to assume that an impure preparation of dibromtyrosine had been obtained from the hydrolysates of Rudistylia gigantea.

THE PHYSIOLOGY OF BROMINE.

In reviewing the results obtained with normal tissues it is evident that, although the figures for most tissues are practically of the same order, thyroid and blood show in each case a slightly higher value than the average figure for all tissues. The relatively high bromine content of blood obviously indicates that its presence in most tissues is merely dependent on their blood supply and is in itself of no particular significance. More reliable data of previous authors, as well as the results recorded, do not indicate an organic combination of bromine in blood.

I have already discussed its relationship to the pituitary gland, and my results confirm those of the more accurate workers, that its presence there is of no functional significance.

It is not possible to state that its presence in the thyroid is without significance, but the data recorded suggest that it is not functionally associated with the active principle of the thyroid nor is it present in any organic combination.

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SUMMARY

Using the method previously described (76), material from a large number of marine plants and animals and from typical land plants and animals has been estimated for bromine; in many cases iodine and total halogen analyses were made on the same material. The results suggest the following conclusions:

Bromine is an invariable constituent of marine algae, but no definite relationship appears to exist between their bromine and iodine contents. Land plants contain considerably less bromine. Environmental conditions, and perhaps selective affinity by the species and cells in the different parts of the individual plant probably determine the actual content of bromine.

All marine species of animals examined contain bromine, but in very variable quantity. Environment and selective cell affinity appear to be the controlling factors.

The thyroid and blood of the mammals completely examined (rat, rabbit and dog) contain amounts of bromine slightly higher than those present in other tissues. Ox, sheep and hog thyroids contain similar amounts. Pituitary tissue contains amounts scarcely if at all greater than most of the other tissues of the mammalian organism. No relationship has been found to exist between the distribution of bromine and iodine in mammalian tissues. Bromine does not appear to be particularly associated with the thyroglobulin of the thyroid.

Bromine is a constant constituent of human tissues. Variations encountered are probably due mainly to difference in intake (in diet and medication). No evidence could be found to support the view that bromine is concerned with pituitary function. The thyroid contains amounts slightly greater than in blood. A functional significance, however, has not been ascertained for this element. No definite relation has been found to exist between the distribution of bromine and chlorine in human tissues.

In agreement with previous investigators, it has been shown that administered bromides are excreted remarkably slowly. The distribution of administered bromide in the various tissues of the rabbit shows a distinct similarity to that of normal chloride, except in the case of the thyroid. No evidence, however, has been obtained to indicate the presence of organically bound bromine in this gland or in the blood.

From the products of hydrolysis of Eudistylis gigantea an impure preparation of a bromine-containing substance (evidently dibromtyrosine) has been made.

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TABLE I.

TYPICAL CHECKS OF PROCEDURE OVER A TEN-MONTH PERIOD.

Date	Iodine present mg.	Chlorine present mg.	Bromine present			Bromine found mg.	Difference mg.
			Inorg. mg.	Org. mg.	Total mg.		
June 11	0.0107	-	0.0332	-	0.0332	0.0327	-0.0005
July 30	-	-	0.0405	-	0.0405	0.0392	-0.0013
Oct. 21	-	-	0.0580	-	0.0580	0.0576	-0.0004
Nov. 20	-	0.120	-	0.0190	0.0190	0.0184	-0.0006
Dec. 27	0.0210	-	0.0819	0.0102	0.0921	0.0912	-0.0009
Jan. 8	-	0.200	0.0432	-	0.0432	0.0422	-0.0010
Feb. 17	0.0150	-	0.0170	-	0.0170	0.0160	-0.0010
Mar. 25	-	-	0.0871	-	0.0871	0.0864	-0.0007

TABLE II.

CONTENT OF BROMINE AND IODINE IN MARINE ALGAE.

Family and Species	Where obtained	Part examined	Amount taken dry material gm.	Bromine Br found mg.	Bromine Br %	Iodine %
Phaeophyceae						
Encoliaceae						
Scytosiphon lomentarius	(e)	Plant	0.3062	0.248	0.081	
			0.4178	0.331	0.081	
				Mean	0.081	0.014
Laminariaceae						
Nereocystis lutkeana	(g)	Frond	0.1071	0.153	0.143	
			0.2438	0.349	0.145	
				Mean	0.143	0.098
Macrocystis pyrifera	(i)	Frond	0.06555	0.052	0.079	
			0.1248	0.101	0.081	
				Mean	0.080	0.200
Fucaceae						
Fucus furcatus	(a)	Plant	0.0864	0.050	0.058	
			0.1954	0.116	0.059	
				Mean	0.0585	0.042
Rhodophyceae						
Rhodophyllidaceae						
Euthora fruticulosa	(a)	Plant	0.0961	0.083	0.087	
			0.2045	0.179	0.088	
				Mean	0.0875	0.053
Dumontiaceae						
Constantinea sitchensis	(a)	Plant	0.10335	0.046	0.043	
			0.23385	0.103	0.044	
				Mean	0.0435	0.019

TABLE III.

CONTENT OF WATER AND BROMINE IN LAND PLANTS.

Family and Species	Where obtained	Part examined	Water %	Amount taken dry material gm.	Bromine Br found mg.	Bromine Br %
Gramineae						
Phleum pratense (Timothy)	(s)	Leaves & stems	38.24	0.5072	0.0096	0.0019
				0.5076	0.0096	0.0019
					Mean	0.0019
Arrhenatherum elatus (Oats)	(q)	Leaves & stems	56.55	0.5084	0.0040	0.0008
				0.5242	0.0048	0.0009
					Mean	0.00085
Zea mais (Indian corn)	(q)	Stem	83.62	0.5023	0.0184	0.0037
				0.5014	0.0184	0.0037
					Mean	0.0037
		Leaves	80.61	0.5166	0.0136	0.0026
				0.5109	0.0136	0.0027
					Mean	0.00265
		Fruit	79.92	0.5096	0.0032	0.0006
				0.5098	0.0032	0.0006
					Mean	0.0006
	(q)	Root	-	0.5049	0.0048	0.0010
				0.5021	0.0032	0.0006
					Mean	0.0008
				Fresh material		
Triticum vulgare (Ceres wheat)	(q)	Root	-	0.5023	0.0032	0.0006
				0.5094	0.0032	0.0006
					Mean	0.0006
		Straw	-	0.5098	0.0048	0.0009
				0.5088	0.0048	0.0009
					Mean	0.0009
		Grain	-	0.5052	0.0008	0.0001
				0.6069	0.0008	0.0001
					Mean	0.0001
Triticum durum - 1933 (Durum wheat)	(r)	Grain	-	0.6151	0.0008	0.0001
				0.5123	0.0008	0.0001
					Mean	0.0001
- 1935				0.6031	0.0024	0.0004
				0.5049	0.0016	0.0003
					Mean	0.00035
Secale cereale (Rye)	(r)		-	0.5282	0.0032	0.0006
				0.6057	0.0032	0.0005
					Mean	0.00035
No.1 & 2 Manitoba Hard Wheat	(n)	Grain	-	0.7075	0.0008	0.0001
				0.5543	0.0008	0.0001
					Mean	0.0001
				0.7501	0.0024	0.0003
				0.5470	0.0024	0.0004
					Mean	0.00035
				0.7135	0.0008	0.0001
				0.5107	0.0008	0.0002
					Mean	0.00015
				0.7572	0.0008	0.0001
				0.5590	0.0008	0.0002
					Mean	0.00015
				0.6637	0.0008	0.0001
				0.6123	0.0016	0.0003
					Mean	0.0002
				0.6051	0.0024	0.0004
				0.7534	0.0024	0.0003
					Mean	0.00035
				0.7085	0.0016	0.0002
				0.6105	0.0016	0.0003
					Mean	0.00025
				0.7142	0.0008	0.0001
				0.5185	0.0008	0.0002
					Mean	0.00015
				0.7397	0.0024	0.0003
				0.6169	0.0008	0.0001
					Mean	0.0002
	(o)			0.7523	0.0008	0.0001
				0.6339	0.0032	0.0005
					Mean	0.0003
			-	0.6116	0.0024	0.0004
				0.7577	0.0024	0.0003
					Mean	0.00035
			-	0.6243	0.0008	0.0001
				0.7082	0.0016	0.0002
					Mean	0.00015

				0.6339	0.0032	0.0005
					Mean	0.0003
				0.6116	0.0024	0.0004
				0.7577	0.0024	0.0003
					Mean	0.00035
				0.6243	0.0008	0.0001
				0.7082	0.0016	0.0002
					Mean	0.00015
				0.7562	0.0016	0.0002
				0.6149	0.0024	0.0004
					Mean	0.0003
				0.6595	0.0032	0.0005
				0.6093	0.0016	0.0003
				0.6112	0.0024	0.0004
				0.5548	0.0024	0.0004
				0.7544	0.0024	0.0003
					Mean	0.00035
				0.6209	0.0024	0.0004
				0.7167	0.0032	0.0004
					Mean	0.0004
	(p)			0.7466	0.0024	0.0003
				0.6100	0.0024	0.0003
				0.6820	0.0056	0.0008
				0.7608	0.0056	0.0007
				0.7500	0.0080	0.0011
			Dry material			
Polygonaceae						
Rheum rhaponticum (Rhubarb)	(q)	Leaves	92.68	0.5061	0.0048	0.0009
				0.5067	0.0048	0.0009
					Mean	0.0009
Chenopodiaceae						
Beta rapa (Red beet)	(q)	Leaves	88.11	0.3049	(none)	<0.00025
				0.5053	(none)	<0.00015
					Mean	<0.00015
		Root	84.36	0.4118	(none)	<0.0002
				0.5073	(none)	<0.00015
					Mean	<0.00015
Cruciferae						
Brassica rapa (Turnip)	(s)	Leaves	83.23	0.5088	0.0216	0.0042
				0.5077	0.0216	0.0043
					Mean	0.00425
		Root	89.49	0.5058	0.0120	0.0024
				0.5052	0.0120	0.0024
					Mean	0.0024
Brassica oleracea (Cabbage)	(s)	Leaves	89.16	0.5176	0.0128	0.0025
				0.5052	0.0128	0.0025
					Mean	0.0025
	(q)		89.50	0.5055	0.0008	0.0002
				0.5059	0.0008	0.0002
					Mean	0.0002
Leguminosea						
Trifolium pratense (Red Clover)	(s)	Leaves & stems	75.36	0.4991	0.0080	0.0016
				0.5012	0.0080	0.0016
					Mean	0.0016
	(s)	Root	64.40	0.5036	0.0032	0.0006
				0.5035	0.0048	0.0009
					Mean	0.00075
Melilotus alba (Sweet clover)	(q)	Leaves & stems	72.75	0.5109	0.0016	0.0003
				0.5070	0.0016	0.0003
					Mean	0.0003
Rosaceae						
Malus communis (Apple)	(s)	Fruit	87.57	0.5089	0.0016	0.0003
				0.5021	0.0016	0.0003
					Mean	0.0003
Pyrophorum communis (Pear)	(s)	Fruit	88.82	0.4997	0.0032	0.0006
				0.4993	0.0032	0.0006
					Mean	0.0006
Rubus idaeus (Raspberry)	(s)	Fruit	86.63	0.5133	0.0048	0.0009
				0.5116	0.0048	0.0009
					Mean	0.0009
Prunus persica (Peach)	(t)	Fruit	88.06	0.5042	(none)	<0.00015
				0.5024	(none)	<0.00015
					Mean	<0.00015
Citrus aurantium (Orange)	(t)	Fruit-edible	89.86	0.5103	0.0032	0.0006
				0.5091	0.0032	0.0006
					Mean	0.0006
		Fruit-pealing	75.42	0.5000	0.0032	0.0006
				0.5020	0.0016	0.0003
					Mean	0.00045
Citrus decumana (Grape-fruit)	(t)	Fruit-edible	91.74	0.5121	0.0048	0.0009
				0.5089	0.0048	0.0009
					Mean	0.0009
		Fruit-pealing	80.96	0.5000	0.0016	0.0003
				0.5005	trace	trace
					Mean	<0.0003
Vitaceae						
Vitis vinifera (Grapes)	(s)	Fruit	87.58	0.5085	0.0048	0.0010
				0.5112	0.0064	0.0012
					Mean	0.0011
	(t)	Fruit	85.37	0.5090	(none)	<0.00015
				0.5083	(none)	<0.00015
					Mean	<0.00015

Brassica oleracea (Cabbage)	(s)	Leaves	89.16	0.5176	0.0128	0.0025
				0.5052	0.0128	0.0025
					Mean	0.0025
	(q)		89.50	0.5055	0.0008	0.0002
				0.5059	0.0008	0.0002
					Mean	0.0002
Leguminoseae						
Trifolium pratense (Red Clover)	(s)	Leaves & stems	75.36	0.4991	0.0080	0.0016
				0.5012	0.0080	0.0016
					Mean	0.0016
	(s)	Root	64.40	0.5036	0.0032	0.0006
				0.5035	0.0048	0.0009
					Mean	0.00075
Melilotus alba (Sweet clover)	(q)	Leaves & stems	72.75	0.5109	0.0016	0.0003
				0.5070	0.0016	0.0003
					Mean	0.0003
Rosaceae						
Malus communis (Apple)	(s)	Fruit	87.57	0.5089	0.0016	0.0003
				0.5021	0.0016	0.0003
					Mean	0.0003
Pyrophorum communis (Pear)	(s)	Fruit	88.82	0.4997	0.0032	0.0006
				0.4993	0.0032	0.0006
					Mean	0.0006
Rubus idaeus (Raspberry)	(s)	Fruit	86.63	0.5133	0.0048	0.0009
				0.5116	0.0048	0.0009
					Mean	0.0009
Prunus persica (Peach)	(t)	Fruit	88.06	0.5042	(none)	<0.00015
				0.5024	(none)	<0.00015
					Mean	<0.00015
Citrus aurantium (Orange)	(t)	Fruit-edible	89.86	0.5103	0.0032	0.0006
				0.5091	0.0032	0.0006
					Mean	0.0006
		Fruit-pealing	75.42	0.5000	0.0032	0.0006
				0.5020	0.0016	0.0003
					Mean	0.00045
Citrus decumana (Grape-fruit)	(t)	Fruit-edible	91.74	0.5121	0.0048	0.0009
				0.5089	0.0048	0.0009
					Mean	0.0009
		Fruit-pealing	80.96	0.5000	0.0016	0.0003
				0.5005	trace	trace
					Mean	<0.0003
Vitaceae						
Vitis vinifera (Grapes)	(s)	Fruit	87.58	0.5085	0.0048	0.0010
				0.5112	0.0064	0.0012
					Mean	0.0011
	(t)	Fruit	85.37	0.5090	(none)	<0.00015
				0.5083	(none)	<0.00015
					Mean	<0.00015
Cornaceae						
Daucus carota (Carrot)	(q)	Leaves & stems	80.54	0.2518	(none)	<0.0003
				0.4050	(none)	<0.0002
					Mean	<0.00025
		Root	89.48	0.3082	(none)	<0.0002
				0.4086	(none)	<0.0002
	(s)	Leaves & stems	77.71	0.5016	0.0981	0.0195
				0.5024	0.0984	0.0196
					Mean	0.01955
		Root	89.58	0.5095	0.0184	0.0036
				0.5123	0.0184	0.0036
					Mean	0.0036
Umbelliferae						
Apium vapaeium (Celery)	(s)	Leaves & stems	87.16	0.5074	0.0880	0.0173
				0.5053	0.0912	0.0180
					Mean	0.01765
Pastinaca sativa (Parsnip)	(q)	Leaves	80.25	0.5080	0.0128	0.0025
				0.5056	0.0128	0.0025
					Mean	0.0025
		Root	77.13	0.5072	0.0064	0.0013
				0.5055	0.0064	0.0013
					Mean	0.0013
Solanaceae						
Solanum tuberosum (Potato)	(q)	Leaves	84.26	0.3077	(none)	<0.00025
				0.40485	(none)	<0.0002
					Mean	<0.0002
		Root	80.21	0.2963	(none)	<0.00025
				0.3436	(none)	<0.00025
					Mean	<0.00025
Lycopersicon esculentum (Tomato)	(q)	Fruit	93.90	0.5045	0.0016	0.0003
				0.5033	0.0016	0.0003
					Mean	0.0003
	(s)	Fruit	95.22	0.5027	0.0064	0.0013
				0.5069	0.0080	0.0015
					Mean	0.0014
Compositae						
Lactuca sativa (Lettuce)	(q)	Leaves	94.51	0.5017	0.0096	0.0019
				0.5012	0.0096	0.0019
					Mean	0.0019
Cucurbitaceae						
Cucurbita sativus (Cucumber)	(q)	Fruit	95.44	0.5024	0.0200	0.0040
				0.5033	0.0200	0.0040
					Mean	0.0040

TABLE IV.

CONTENT OF BROMINE AND IODINE IN MARINE ANIMALS.

Family and Species	Where obtained	Part examined	Amount taken dry material gm.	Bromine: Br found mg.	Bromine: Br %	Iodine %
Phylum Porifera						
Monaxonida						
Myxilla parasitica	(b)	Animal	0.0836	0.020	0.024	
			0.2343	0.057	0.025	
				Mean	0.0245	0.010
Esperella adhaerens	(b)	Animal	0.08625	0.017	0.019	
			0.20475	0.040	0.020	
				Mean	0.0195	0.015
Hexactinellida						
Bathydorus dawsoni	(b)	Animal	0.0563	0.0040	0.0071	
			0.0758	0.0056	0.0074	
				Mean	0.00725	0.009
Rhabdocalyptus dowlingii	(b)	Animal	0.08725	0.0016	0.0018	
			0.16445	0.0032	0.0019	
				Mean	0.00185	0.014
Phylum Coelenterata						
Hydrozoa						
Aequorea forskalia	(d)	Animal	0.18725	0.214	0.114	
			0.0759	0.086	0.113	
				Mean	0.1135	0.000
Scyphozoa						
Aurelia flavidula	(d)	Animal	0.0856	0.164	0.192	
			0.0847	0.163	0.193	
				Mean	0.1925	0.000
Actinozoa						
Matridium marginatum	(a)	Animal	0.08445	0.030	0.035	
			0.1737	0.062	0.036	
				Mean	0.0355	0.000
Ctenophora						
Pleurobrachia (sp?)	(a)	Animal	0.07815	0.148	0.189	
			0.1174	0.212	0.189	
				Mean	0.189	0.000
(Verticillate fan coral)	(j)	Whole coral	0.2042	0.718	0.352	
? Caligorgia			0.40455	1.442	0.356	
				Mean	0.354	0.057
		Skeleton	0.04505	0.394	0.874	
			0.07785	0.683	0.878	
				Mean	0.876	0.099
Phylum Vermes						
Eunicea						
Diopatra (? californica)	(h)	Inner tubes	0.10415	0.121	0.116	
			0.2001	0.239	0.119	
				Mean	0.1175	0.122
		Interm. tubes	0.10155	0.123	0.121	
			0.2057	0.251	0.122	
				Mean	0.1215	0.128
		Outer tubes	0.30455	0.121	0.039	
			0.4027	0.158	0.039	
				Mean	0.039	0.041
Chaetopteridæ						
Chaetopterus (sp ?)	(e)	Inner tubes	0.1035	0.330	0.318	
			0.0714	0.230	0.320	
				Mean	0.319	0.450
		Interm. tubes	0.1257	0.297	0.236	
			0.18175	0.416	0.229	
				Mean	0.2325	0.333
		Outer tubes	0.2032	0.305	0.150	
			0.1505	0.229	0.152	
				Mean	0.151	0.212
		Tube-ends	0.3024	0.145	0.048	
			0.1067	0.049	0.046	
				Mean	0.047	0.096
Sabellidæ						
Eudistylia gigantea	(d)	Worm	0.3080	0.062	0.020	
			0.4007	0.078	0.019	
				Mean	0.0195	0.030
		Inner tube	0.0723	0.029	0.040	
			0.0507	0.021	0.040	
				Mean	0.040	0.616
		Interm. tube	0.05425	0.020	0.036	
			0.0729	0.027	0.037	
			0.20805	0.078	0.038	
				Mean	0.037	0.606

		Inner tube	0.0723	0.029	0.040	
			0.0507	0.021	0.040	
					Mean 0.040	0.616
		Interm. tube	0.05425	0.020	0.036	
			0.0729	0.027	0.037	
			0.20805	0.078	0.038	
					Mean 0.037	0.606
	(a)	Outer tube	0.1054	0.086	0.082	
			0.14025	0.116	0.083	
			0.2044	0.171	0.084	
					Mean 0.083	0.507
		Tube	0.0899	0.062	0.069	
			0.1734	0.119	0.068	
					Mean 0.0685	0.572
Bispira polymorpha	(a)	Tube	0.07615	0.103	0.136	
			0.1406	0.190	0.135	
					Mean 0.1355	0.656
			0.05545	0.074	0.134	
			0.1041	0.141	0.134	
					Mean 0.134	0.698
Phoronida						
Phoronopsis harmeri	(c)	Outer tube	0.3103	0.0061	0.0020	
			0.45745	0.0086	0.0019	
					Mean 0.00195	0.009
Phylum Mollusca						
Schizotherus nuttalli (Horse-clam)	(c)	Dermis of foot	0.2028	0.205	0.101	
			0.17275	0.179	0.104	
					Mean 0.1025	0.092
	(c)		0.2102	0.215	0.102	
			0.30445	0.301	0.099	
					Mean 0.1005	0.103
Mytilus edulis (Mussel)	(a)	Byssus	0.2021	0.274	0.135	0.042
Polynices lewisii (Whelk)	(c)	Opercula	0.17155	0.660	0.385	
			0.06755	0.256	0.379	
					Mean 0.382	0.030
Phylum Chordata						
Sub-phylum Tunicata						
Tethyidae						
Pyura haustor	(h)	Test	0.5082	0.502	0.099	
			0.5042	0.493	0.098	
					Mean 0.0985	0.216
	(h)		0.50695	0.809	0.160	0.247
	(c)		0.5020	0.278	0.055	
			0.5006	0.251	0.051	
					Mean 0.053	0.027
Tethyum igaboja	(b)	Test	0.1397	0.323	0.231	
			0.2103	0.472	0.225	
					Mean 0.228	0.169
Styelidae						
Cnemidocarpa jeannae	(a)		0.2261	0.301	0.153	0.106
Phalusiidae						
Ascidiopsis paratropa	(f)		0.2810	0.579	0.206	
			0.1935	0.405	0.209	
					Mean 0.2075	0.010
Phylum Chordata						
Sub-Phylum Vertebrata						
Raia clavata	(m)	Thyroid	0.1701	0.080	0.047	
			0.1760	0.082	0.046	
					Mean 0.0465	0.438
			0.2032	0.110	0.054	
			0.19085	0.100	0.052	
					Mean 0.053	0.327
			0.2080	0.216	0.104	
			0.22755	0.241	0.105	
					Mean 0.1045	0.283
Scyllium canicula	(m)		0.2034	0.102	0.050	
			0.2088	0.107	0.051	
					Mean 0.0505	0.719
			0.2060	0.165	0.080	
			0.22325	0.175	0.078	
					Mean 0.079	1.160
Acanthias vulgaris	(k)		0.1631	0.021	0.013	
			0.13675	0.018	0.013	
					Mean 0.013	0.133
Squalus sucklii	(a)		0.1119	0.037	0.033	0.216
			0.08445	0.033	0.039	0.185
Hydrolagus colliei	(a)	Egg-case	0.20175	0.074	0.037	
			0.15355	0.058	0.038	
					Mean 0.0375	0.029

TABLE V.

CONTENT OF WATER AND BROMINE IN MAMMALIAN MATERIAL.

Animal	Part examined	Water %	Amount taken dry material. gm.	Bromine: Br found mg.	Bromine: Br %	Calculated to Br in fresh material %
Albino rats	Blood	80.39	0.5090	0.0136	0.0027	
			0.3580	0.0120	0.0033	
				Mean	0.0030	0.00059
	Bone	43.26	0.5093	0.0032	0.0006	0.00034
	Cerebellum	76.74	0.4491	0.0056	0.0012	
			0.4088	0.0056	0.0013	
				Mean	0.00125	0.00028
	Cerebrum	79.62	0.4992	trace	trace	
			0.5004	trace	trace	
				Mean	trace	trace
	Eye	81.02	0.3803	0.0048	0.0013	0.00025
	Heart	77.34	0.5026	0.0072	0.0014	
			0.3804	0.0048	0.0013	
				Mean	0.00135	0.000295
	Large Intestine	78.60	0.5027	0.0048	0.0010	0.000215
	Small Intestine	78.18	0.5026	0.0032	0.0006	
			0.5026	0.0032	0.0006	
				Mean	0.0006	0.00013
	Kidney	83.77	0.5042	0.0056	0.0011	
			0.5052	0.0056	0.0011	
				Mean	0.0011	0.00018
	Liver	74.97	0.4991	0.0024	0.0005	
			0.5015	0.0032	0.0006	
				Mean	0.00055	0.000125
	Lung	79.32	0.5090	0.0104	0.0020	
			0.5102	0.0096	0.0019	
				Mean	0.00195	0.00039
	Medulla	71.23	0.2833	0.0048	0.0017	0.00050
	Muscle	74.52	0.4979	0.0032	0.0006	
			0.5021	0.0024	0.0005	
				Mean	0.00055	0.000125
	Pancreas	65.19	0.4990	0.0032	0.0006	
			0.5088	0.0040	0.0008	
				Mean	0.0007	0.00024
	Skin	58.68	0.5138	0.0040	0.0008	
			0.5147	0.0048	0.0008	
				Mean	0.0008	0.00033
	Spleen	77.07	0.5003	0.0040	0.0008	0.000185
	Stomach	78.99	0.5090	0.0080	0.0016	
			0.5073	0.0064	0.0013	
				Mean	0.00145	0.00030
	Testes	85.81	0.5137	0.0091	0.0018	
			0.5138	0.0109	0.0021	
				Mean	0.00195	0.00027
	Uterus	73.55	0.1904	0.0040	0.0021	0.00056
	Hair	Fresh material	0.4965	0.0019	-	0.0004
Rabbits	Adrenal	70.48	0.2918	0.0032	0.0011	0.00032
	Blood	81.71	0.5150	0.0144	0.0028	
			0.5115	0.0144	0.0028	
				Mean	0.0028	0.00051
	Bone	32.72	0.5143	0.0048	0.0009	
			0.5411	0.0048	0.0009	
				Mean	0.0009	0.000605
	Bone-Marrow	52.62	0.5012	0.0032	0.0006	
			0.5138	0.0032	0.0006	
				Mean	0.0006	0.000285
	Cartilage	67.39	0.5063	0.0064	0.0013	
			0.5140	0.0064	0.0013	
				Mean	0.0013	0.00043
	Cerebellum	79.73	0.5054	0.0016	0.0003	
			0.5067	0.0016	0.0003	
				Mean	0.0003	0.00006
	Cerebrum	78.62	0.5096	0.0048	0.0009	0.00019
	Eye	87.68	0.5155	0.0120	0.0023	
			0.5125	0.0120	0.0023	
				Mean	0.0023	0.00028
	Heart	78.16	0.5126	0.0016	0.0003	
			0.5206	0.0016	0.0003	
				Mean	0.0003	0.000065
	Kidney	78.95	0.5112	0.0032	0.0006	
			0.5088	0.0032	0.0006	
				Mean	0.0006	0.00013
	Liver	75.43	0.5179	0.0016	0.0003	
			0.5083	0.0016	0.0003	
				Mean	0.0003	0.00007
	Lung	80.20	0.5049	0.0032	0.0006	
			0.5059	0.0032	0.0006	
				Mean	0.0006	0.00012

	Skin	69.05	0.5707	0.0016	0.0005	
			0.5745	0.0016	0.0003	
				Mean	0.0003	0.00009
	Spleen	77.96	0.4919	0.0032	0.0006	0.00013
	Stomach	80.51	0.5033	0.0064	0.0013	
			0.5055	0.0064	0.0013	
				Mean	0.0013	0.00025
	Testes	79.91	0.5105	0.0072	0.0014	
			0.5073	0.0056	0.0011	
				Mean	0.00125	0.00024
	Thymus	51.78	0.5136	0.0016	0.0003	
			0.3618	0.0016	0.0004	
				Mean	0.00035	0.00014
	Uterus	80.26	0.5022	0.0224	0.0045	
			0.5149	0.0232	0.0045	
				Mean	0.0045	0.00089
	Hair	Fresh	0.5114	0.0032	-	0.0006
		material	0.5115	0.0032	-	0.0006
				Mean	-	0.0006
	Ovary	52.12	0.4780	0.0072	0.0015	0.00072
	Pituitary	74.21	0.2562	0.0040	0.0016	0.00041
	Thyroid	71.30	0.1077	0.0032	0.0030	
			0.3549	0.0104	0.0029	
				Mean	0.00295	0.00083
Dog 1.	Adrenal	63.85	0.4993	0.0032	0.0006	0.000215
	Bladder-bile	53.49	0.5034	0.0016	0.0003	
			0.4993	0.0016	0.0003	
				Mean	0.0003	0.00014
	Blood	76.21	0.5018	0.0184	0.0037	
			0.5023	0.0200	0.0040	
				Mean	0.00385	0.000905
	Bone	22.67	0.5067	0.0040	0.0008	
			0.5016	0.0040	0.0008	
				Mean	0.0008	0.00062
	Bone-Marrow	33.81	0.5100	0.0048	0.0009	
			0.4228	0.0048	0.0011	
				Mean	0.0010	0.00066
	Cerebellum	79.03	0.5083	0.0032	0.0006	
			0.5115	0.0040	0.0008	
				Mean	0.0007	0.000145
	Cerebrum	79.37	0.5118	0.0048	0.0009	
			0.5077	0.0040	0.0009	
				Mean	0.0009	0.000165
	Eye	88.25	0.5013	0.0552	0.0110	
			0.5037	0.0512	0.0102	
				Mean	0.0106	0.001245
	Gall-bladder	68.07	0.5075	0.0040	0.0008	0.000255
	Heart	76.06	0.5029	0.0040	0.0008	
			0.5047	0.0040	0.0008	
				Mean	0.0008	0.00019
	Large Intestine	78.80	0.5066	0.0096	0.0019	
			0.5054	0.0112	0.0022	
				Mean	0.00205	0.000425
	Small Intestine	81.37	0.5012	0.0096	0.0019	
			0.5033	0.0096	0.0019	
				Mean	0.0019	0.000355
	Kidney	79.03	0.5123	0.0168	0.0033	
			0.5083	0.0168	0.0033	
				Mean	0.0033	0.00069
	Liver	77.62	0.5006	0.0104	0.0021	
			0.5006	0.0104	0.0021	
				Mean	0.0021	0.00047
	Lung	78.98	0.5090	0.0104	0.0020	
			0.5082	0.0104	0.0020	
				Mean	0.0020	0.00042
	Muscle	74.02	0.5074	0.0016	0.0003	
			0.5087	0.0016	0.0003	
				Mean	0.0003	0.00008
	Ovary	72.34	0.1921	0.0024	0.0012	0.00033
	Pancreas	74.54	0.5080	0.0048	0.0009	
			0.5054	0.0040	0.0008	
				Mean	0.00085	0.000205
	Pituitary	79.88	0.0192	(none)	<0.004	<0.0008
	Skin	47.76	0.5103	0.0096	0.0019	
			0.5203	0.0104	0.0020	
				Mean	0.00195	0.00099
	Spleen	79.17	0.5101	0.0120	0.0024	
			0.5025	0.0120	0.0024	
				Mean	0.0024	0.00050
	Stomach	79.12	0.5079	0.0144	0.0028	
			0.5120	0.0128	0.0025	
				Mean	0.00265	0.00054
	Thymus	69.53	0.5104	0.0072	0.0014	
			0.2696	0.0040	0.0015	
				Mean	0.00145	0.000425
	Thyroid	73.08	0.4180	0.0184	0.0044	
			0.2577	0.0104	0.0040	
				Mean	0.0042	0.00113
	Uterus	77.04	0.4211	0.0080	0.0019	0.000435
	Hair	Fresh	0.5058	0.0048	-	0.0009
		material	0.5105	0.0032	-	0.0006
				Mean	-	0.00075
Dog 2.	Thyroid	-	0.2308	0.0104	0.0045	
			0.3625	0.0152	0.0042	
				Mean	0.00435	

TABLE VI.

CONTENT OF WATER, BROMINE AND IODINE IN NORMAL ENDOCRINE MATERIAL.

Material	Animal	Source	Water %	Amount taken dry material gm.	Bromine: Br found mg.	Bromine: Br %	Calculated to Br in fresh material %	Iodine dry material %
Thyroid	Cattle	Winnipeg	79.21	0.10575	0.0048	0.0045		0.061
				0.2061	0.0096	0.0046		0.063
					Mean	0.00455	0.000935	0.062
		Commercial	-	0.2969	0.029	0.009		0.430
				0.4074	0.036	0.009		
					Mean	0.009	-	0.430
	Hog	Northern U.S.A.	-	0.2037	0.094	0.046		0.282
				0.29955	0.131	0.044		0.285
					Mean	0.045	-	0.2835
		Central U.S.A.	-	0.20145	0.074	0.036		0.373
				0.29855	0.105	0.035		0.373
					Mean	0.0355	-	0.373
		Southern U.S.A.	-	0.2038	0.069	0.033		0.667
				0.4039	0.144	0.035		0.668
					Mean	0.034	-	0.6675
	?	Commercial	-	0.22265	0.050	0.022		0.151
				0.42125	0.091	0.022		
					Mean	0.022	-	0.151
	Sheep	U.S.A.	-	0.22325	0.045	0.020		0.358
				0.42315	0.086	0.020		
					Mean	0.020	-	0.358
Pituitary ant.lobe	Cattle	Winnipeg	75.94	0.1126	0.0032	0.0029		
				0.3575	0.0104	0.0029		
					Mean	0.0029	0.00070	-
Pituitary whole gland	Cattle	U.S.A.	-	0.39975	0.0264	0.0066		0.014
				0.50225	0.0360	0.0071		0.013
					Mean	0.00685	-	0.0135
Pituitary post. lobe	Cattle	Winnipeg	77.97	0.1084	0.0032	0.0030		
				0.35885	0.0145	0.0040		
					Mean	0.0035	0.00077	-
		U.S.A.	-	0.4020	0.0038	0.0009		trace
				0.5027	0.0045	0.0009		
					Mean	0.0009	-	trace
Adrenal medulla	Cattle	Winnipeg	78.97	0.42535	0.0080	0.0019	0.00040	-
Adrenal cortex	Cattle	Winnipeg	77.27	0.42675	0.0032	0.0008		
				0.5029	0.0048	0.0009		
					Mean	0.00085	0.00018	-
			-	0.3353	(none)	<0.0003		
				0.11385	(none)	<0.0006		
					Mean	<0.0003	-	-
			-	0.5118	0.0096	0.0019		
				0.4023	0.0080	0.0020		
					Mean	0.00195	-	-
Ovarian residue	?	Commercial	-	0.4044	0.0064	0.0016		0.014
				0.5127	0.0080	0.0016		
					Mean	0.0016	-	0.014
Corpora lutea	?	Commercial	-	0.40715	0.0064	0.0016		0.014
				0.5021	0.0064	0.0013		
					Mean	0.00145	-	0.014

TABLE VII.

BROMINE AND IODINE ANALYSES OF THYROID FRACTIONS.

Material	Water %	Amount taken dry material. gm.	Bromine: Br found mg.	Bromine: Br %	Calculated to Br in fresh material %	Iodine dry material %
Thyroid - normal (Beef)	74.08	0.11245	0.0040	0.0036		0.256
		0.21005	0.0096	0.0046		0.256
			Mean	0.0041	0.00106	0.256
residue left after) extn. with 1 % NaCl)	82.96	0.1075	0.0040	0.0037		0.042
		0.2047	0.0088	0.0043		0.044
			Mean	0.0040	0.00068	0.043
- normal (Beef)	-	0.15535	0.0064	0.0041		
		0.3565	0.0160	0.0045		
			Mean	0.0043	-	-
residue left after) extn. with 1 % NaCl)	-	0.1537	0.0024	0.0016		
		0.3521	0.0072	0.0020		
			Mean	0.0018	-	-
Thyroid - normal (Hog)	75.17	0.1020	0.0144	0.0141		0.199
		0.31185	0.0456	0.0146		0.198
			Mean	0.01435	0.00356	0.1985
residue left after) extn. with 1% NaCl)	85.26	0.11445	0.0072	0.0063		0.077
		0.3181	0.0208	0.0065		0.084
			Mean	0.0064	0.00094	0.0805
Thyroglobulin, pure, 1926 (Beef)	-	0.3192	0.066	0.021		0.619
		0.41855	0.088	0.021		
			Mean	0.021	-	0.619
	-	0.3216	0.048	0.015		0.634
		0.4195	0.068	0.016		0.636
			Mean	0.0155	-	0.635
coitrous	-	0.3215	0.0080	0.0023		0.282
		0.48185	0.0112	0.0023		0.276
			Mean	0.0023	-	0.279
Thyroglobulin, pure, 1926 (Hog)	-	0.47025	0.0350	0.0074		0.443
		0.4921	0.0380	0.0077		
			Mean	0.00755	-	0.443
coitrous	-	0.48975	0.0270	0.0055		0.320
		0.4994	0.0300	0.0060		
			Mean	0.00575	-	0.320
Thyroid nucleoprotein, 1926	-	0.5037	(none)	<0.00015		0.025
		0.4987	(none)	<0.00015		0.023
			Mean	<0.00015	-	0.024

TABLE VIII.

CONTENT OF BROMINE IN MISCELLANEOUS MATERIAL.

Material	Amount taken fresh material c.c.	Bromine found mg.	Bromine gm. per 100 c.c.
Dairy milk	5.00	0.0048	0.000096
Wheat germ oil	3.00	(none)	< 0.00003
	gm.		%
White bread (wrapped)	0.6159	0.0019	0.0003
(unwrapped)	0.6083	0.0040	0.0007
Dog biscuits	0.5143	trace	trace
Wheat germ flower	0.5117	0.0008	0.0001
Virginia cigarette tobacco	0.5069	0.0059	0.0012

TABLE X.

BROMINE CONTENT OF PLANT MATERIAL

Material	Bromine in dried material	
	Damiens (25,26)	Neufeld (N)
	%	%
Edible fungi (9 species)	0.00019 - 0.00362	-
Flowering plants		
Gramineae (Wheat)	0.00021	0.0001 - 0.0011 *
(Rye)	0.00019	0.0005 *
(Other cereals - 5 species)	traces - 0.00056	-
Liliaceae (Garlic, onion, eschalot, leek)	0.00010 - 0.00052	-
(Asparagus)	0.00202	-
Urticaceae (Hemp)	0.00021 - 0.00023	-
Polygonaceae (Rhubarb)	0.00075	0.0009
Chenopodiaceae (Beet-root)	0.00037 - 0.00055	(none)
Cruciferae (Turnip)	0.00031 - 0.00089	0.0024
(Cabbage)	0.00045	0.0002 - 0.0025
(Radish, cauliflower)	0.00067 - 0.00083	-
Leguminosae (8 species)	traces - 0.00102	-
Umbelliferae (Celery)	0.00038 - 0.00047	0.01765
Cornaceae (Carrot)	0.00039	(none) - 0.0036
Labiatae (Woundwort)	0.00061	-
Solanaceae (Potato)	0.00027 - 0.00143	(none)
Compositae (2 species of artichoke)	0.00062 - 0.00098	-
Fruits		
Peach	traces - 0.00047	(none)
Apple	traces	0.0003
Raspberry	tr	
Peach	traces - 0.00047	(none)
Apple	traces	0.0003
Raspberry	traces	0.0009
Orange	0.00032	0.0006
Grape	0.000195	(none) - 0.0011
Tomato	0.00095 - 0.00534	0.0003 - 0.0014
10 other species	(none) - 0.00071	-
Muskmelon	0.00945	-
Watermelon	0.0262	-
Miscellaneous related material		
Wheat bread	0.00009 - 0.00061	0.0003 - 0.0007*
Wheat flower	0.00009 - 0.00012	-
Wheat germ bread	0.00068	-

* Fresh material.

TABLE XI

BROMINE CONTENT OF ANIMAL FLUIDS (VARIOUS AUTHORS)

MG. PER 100 CC.

Animal	Whole blood	Blood serum	Red corpuscles	Bile	Urine
Rat	(2.2) (37) 0.59 (N)	-	-	-	-
Rabbit	0.51 (N)	-	-	-	-
Guinea pig	(2.3) (37)	-	-	-	-
Dog	(0.63-1.72) (9)	(0.71-0.83) (9)	-	0.08 (24)	0.05 (24)
	0.42 (24)	0.60 (24)	-	0.14 (N)	-
	0.91 (N)	-	-	-	-
Hog	(1.3) (37)	0.75-1.25 (29)	-	-	-
Sheep	(1.8) (37)	-	-	-	-
Cow	(11.2) (50)	-	0.90 (N)	-	(2-3) (62)
	(1.7) (37)	-	-	-	-
	(0.95) (54)	-	-	-	-
	(none) (62)	-	-	-	-
	0.52 (24)	-	-	-	-
	0.71-1.33 (N)	-	-	-	-
Horse	(1.05) (54)	-	-	-	-

TABLE XII

BROMINE CONTENT OF ANIMAL TISSUES (VARIOUS AUTHORS)

MG. PER 100 GM. FRESH TISSUE

Tissue	Rat (N)	Rabbit (N)	Dog (N)	Dog (22,23,24)	Dog (9)	Hog (29)	Cow (various)
Adrenal	-	0.32	0.215	-	3.3 -5.0	0.368	0.15 (24)
Aorta	-	-	-	-	1.66-2.5	-	-
Bone	0.34	0.605	0.62	-	-	-	-
Bone-marrow	-	0.285	0.66	-	-	-	-
Cartilage	-	0.43	-	-	0.77	-	-
Cerebellum	0.28	0.06	0.145	0.20	0.55-0.90	-	-
Cerebrum	trace	0.19	0.165	-	0.53-1.25	0.191 - 0.192	(19.3) (50) (0.02)(62)
Eye	0.25	0.28	1.245	-	-	-	-
Fatty depots	-	-	-	-	0.63-0.71	-	-
Gall bladder	-	-	0.255	-	-	-	-
Hair	0.4	0.6	0.7	-	-	-	-
Heart	0.295	0.065	0.19	0.16	0.55-0.63	-	(none) (62)
Large intestine	0.215	-	0.425	-	-	-	-
Small intestine	0.13	-	0.355	-	0.50-0.55	-	(26.8) (50)
Kidney	0.18	0.13	0.69	0.40	0.59-0.83	0.361 - 0.445	(20.9) (50) (none) (62)
Liver	0.125	0.07	0.47	0.25	0.40-0.63	0.213 - 0.295	(10.1) (50) (none) (62) (0.559)(48)
Lung	0.39	0.12	0.42	0.40	0.71-0.83	0.397 - 0.55	(22.9) (50) 0.42 (24)
Medulla	0.50	0.10	-	-	-	-	-
Muscle	0.125	0.07	0.08	0.10	0.50	-	(22.1) (50)
Ovary	-	0.72	0.33	-	-	0.647	(0.836)(48)
Parathyroid	-	-	-	-	-	-	(5.887)(48)
Pancreas	0.24	-	0.205	-	0.55-0.63	0.259 - 0.265	-
Pituitary	-	0.41	-	-	12.5	0.270	(none-0.23)(62)
Pituitary ant.lobe	-	-	-	-	-	-	(8.716) (48) (15-30) (120)
Pituitary post.lobe	-	-	-	-	-	-	(0.079) (48)
Skin	0.33	0.09	0.99	-	0.37-0.43	-	-
Spleen	0.185	0.13	0.50	0.41	0.63-0.71	-	(21.4) (50) (none) (62)
Stomach	0.30	0.25	0.54	-	0.60-0.77	-	(22.5) (50)
Testes	0.27	0.24	-	0.53	0.63-0.71	0.334	(20.3) (50) (0.85-2.2) (54)
Ad-testes	-	-	-	-	-	-	(0.9-1) (54)
Plexus pampiniformis	-	-	-	-	-	-	(0.95-2.8) (54)
Thymus	-	0.14	0.425	-	-	-	(21.) (50)
Thyroid	-	0.63	1.13	-	0.84-1.45	-	(35.) (50) (6.691) (48)
Trachea	-	-	-	0.20	-	-	(0.07-3.0) (62) -
Uterus	0.56	0.89	0.435	-	-	-	-

TABLE XIII.

BROMINE CONTENT OF FRESH HUMAN TISSUES (VARIOUS AUTHORS).

MG. PER 100 G.

Tissue	Damiens (24)	Dixon (29)	Voko (102)	Neufeld
Adrenal	-	-	0.33 - 0.66	0.22
Blood	0.52	0.28 - 1.64	0.15 - 0.35	0.62 - 1.01
Kidney	0.25	-	0.27	0.335- 0.82
Liver	0.18 - 0.37	-	0.17	0.04 - 0.43
Lung	0.14 - 0.28	-	-	0.33 - 0.715
Pituitary	-	0.42 - 2.39	-	0.12 - 0.155
Spleen	-	-	0.24 - 0.33	0.225- 0.58
Thyroid	-	-	0.48	1.78 - 2.325

TABLE XIV.

BROMINE IN HYDROZOA.

Species	Bromine per 100 gm. fresh substance %	Water per 100 gm. %	Bromine in equivalent weight of sea-water. gm.
Aequorea forskalia	0.0062	94.6	0.0037
Aurelia flavidula	0.0121	93.7	0.0037
Matridium marginatum	0.0034	90.7	0.0036

TABLE XV.

ANALYSES OF HUMAN THYROID MATERIAL.

IN MG.%

	Water	Bromine		Iodine		Total halogen		Equiatomic distribution		
		dry tissue	fresh tissue	dry tissue	fresh tissue	dry tissue	fresh tissue	Br	I	Cl
Normal	76.74	10.1	2.33	169.	39.3	678.5	157.8	1 :	10.6 :	141.2
Normal	77.77	8.1	1.78	107.	23.8	660.	146.8	1 :	8.4 :	176.2
Graves' disease	81.94	50.3	9.08	264.5	47.8	752.5	135.9	1 :	8.4 :	29.5
Graves' disease	86.30	12.45	1.71	186.5	25.6	760.5	104.2	1 :	9.6 :	127.0
Graves' disease long standing	87.97	6.8	0.82	83.	10.0	597.	71.8	1 :	8.0 :	188.6
Pressure symptoms nontoxic goitre	82.58	13.05	2.28	43.5	7.6	4157.	724.	1 :	2.1 :	713.2

TABLE XVI.

THE EXCRETION OF BROMINE FOLLOWING THE ADMINISTRATION OF BROMIDE.

Days	Rabbit 1.				Rabbit 2.			
	Bromide injected mg.	Bromide injected to date mg.	Urine excretion mg.	Faeces excretion mg.	Bromide injected mg.	Bromide injected to date mg.	Urine excretion mg.	Faeces excretion mg.
1.	53.89	53.89	0.74	0.16	46.36	46.36	0.26	0.11
2.	53.89	107.784	1.47	0.28	46.36	92.72	1.12	0.45
3.	53.89	161.68	3.36	0.45	46.36	139.08	3.06	0.05
4.	53.89	215.57	6.44	0.24	46.36	185.44	4.90	0.32
5.	53.89	269.46	8.8	0.33	46.36	231.80	10.5	0.76
6.	53.89	323.35	14.0	0.18	46.36	278.16	6.56	0.02
7.	53.89	377.24	14.0	1.15	46.36	324.52	10.9	0.85
8.	53.89	431.14	18.2	0.21	46.36	370.88	5.33	2.35
9.	53.89	485.03	16.4	1.99	46.36	417.24	10.2	2.18
10.	53.89	538.92	19.2	2.31	46.36	463.60	15.6	3.06
11.			19.2	5.1			27.2	2.31
12.							42.4	2.46
13.							37.1	2.86
14.							28.0	2.95

TABLE XVII.

THE WATER AND BROMINE CONTENT OF TISSUES FOLLOWING THE ADMINISTRATION OF BROMIDE.

Organ	Rabbit 1.			Rabbit 2.			Cameron & Walton (18)
	Water content %	Br fresh material mg.%	Br in whole organ mg.	Water content %	Br fresh material mg.%	Br in whole organ mg.	Normal Cl content Dog mg. %
Adrenal	62.99	23.96	0.116	62.69	5.91	0.029	124
Aorta	55.69	31.50	0.109	60.72	15.20	0.053	
Bile	75.02	1.33	0.006	78.04	3.45	0.038	
Blood	81.56	36.37	80.025	80.79	19.03	40.91	
Bone	15.95	11.09	20.525	18.34	6.78	10.79	103
Bone-marrow	33.18	16.07	1.607	30.80	3.25	0.487	
Cartilage	63.57	29.65	14.827				190
Cerebellum	80.05	13.75	0.136	77.71	5.43	0.093	148
Cerebrum	80.07	12.35	0.742	79.96	4.49	0.293	
Eyes	85.62	32.45	1.821	87.14	18.39	1.200	
Gall bladder	78.91	12.25	0.017	78.27	10.67	0.029	
Hair	7.21	12.34	4.936	10.85	3.83	1.084	
Heart	73.70	20.60	1.145	76.52	6.34	0.311	119
Large intestine	80.65	10.08	4.055	84.16	3.96	0.136	
Small intestine	79.64	16.31	4.686	82.68	7.25	0.356	132
Kidney	77.31	31.76	4.054	78.32	13.16	1.719	251
Liver	73.39	17.52	12.19	73.30	8.09	5.23	136
Lung	78.84	27.61	1.767	79.50	12.59	0.956	230
Medulla	75.54	12.06	1.085	71.56	6.75	0.540	118
Muscle	75.77	4.93	62.86	75.99	2.21	22.33	67
Oesophagus	98.41	16.41	0.210	71.64	7.56	0.109	
Pancreas	59.41	22.06	0.225	66.54	10.30	0.094	138
Pituitary	79.57	18.16	0.005	80.07	7.60	0.002	
Skin	62.04	39.97	79.94	68.54	17.77	30.57	
Spleen	76.46	15.83	0.140	78.36	10.08	0.102	171
Stomach	78.76	31.37	6.44	78.29	8.51	1.711	160
Testes	67.98	39.25	2.774	82.89	10.37	0.539	187
Thymus	43.86	33.26	0.684	54.57	8.02	0.215	
Thyroid	72.36	42.47	0.294	66.67	21.86	0.076	161
Trachea	54.33	27.03	0.308	60.45	11.98	0.120	