XI. BROMINE IN THE TISSUES.

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THE findings of Zondek and Bier [1932, 1; 1933] with respect to the low blood bromine values of manic depressive psychotics have been criticised by many [Fleishhacker and Scheiderer, 1932; 1933; Hahn, 1933; Holtz and Roggenbau, 1933; and Dixon, 1934] on the ground that the method of bromine determination employed, that of Pincussen and Roman [1929], is untrustworthy. Since other investigators using the same chemical technique have confirmed these results on manic depressives [Urechia and Retezeanu, 1933; Sacristan and Peraita, 1933] or have found very constant values for normal blood [Kuranami, 1932] it seemed desirable to repeat these determinations, using the method of Dixon [1934]. It was thought also that confirmation was needed for the theory that a bromine-containing compound was stored in the pituitary which circulated in the blood causing sleep and sedative effects [Zondek and Bier, 1932, 2]. This theory followed on the earlier findings of Bernhardt and Ucko [1926] who claimed that the pituitary had a bromine content 15-30 times that found in blood.

It was therefore decided to investigate the variability of normal human blood bromine values. The results below were obtained with donors including medical officers, laboratory co-workers, *etc.*

D.	0.53 mg./100 ml.	McC.	0.986 mg./100 ml.
N.C.	0.42 ,,	C.N.	1.358 ,,
Hst.	0.63 ,,	C.	0.78 ,,
How.	0.71 ,,	P.	0.84 ,,
Mort.	0.69 ,,	I.	0.39 ,,

The values are so widely divergent that it might be thought, in view of the constancy of the blood bromine claimed by other workers, that errors had been made in the experimental technique or in the selection of the blood donors. Assuming that the experimental technique is correct, the possibility that the blood donors had been subjected to some form of bromide therapy must still be excluded. Since it is known that any ingested bromide is excreted from the body very slowly especially if the food is low in chloride [Hastings *et al.* 1932; Palmer and Clark, 1932] great care was exercised to ensure that the blood donors had received no bromide or iodide for over six months previous to the taking of the blood sample. This could be done easily in the normal series. In the case of inmates of mental hospitals where case-books were the only record there was necessarily less certainty. It was further found almost impossible to rely on blood samples from in-patients of a general hospital. Thus a group of 12 in-patients at King's College Hospital, untreated with bromide since admission, were investigated with respect to their blood bromine, using Walter's [1924] approximate clinical method for the determinations. In two cases the blood bromine was over 10 mg./100 ml. (The clinical method does not detect values of

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1 mg./100 ml.) These facts indicate some of the difficulties in the interpretation of values obtained for the bromine content of blood or tissues if the possibility of bromide dosage is not considered. They also make it probable that the variations in normal series are due to differences in bromine intake.

Little systematic work on the bromine content of common foodstuffs has been done, although von Damiens and Blaignan [1931; 1932], using methods for bromine estimation which are not grossly inaccurate, have demonstrated its presence in varying amounts throughout the vegetable kingdom. The abbreviated table below, taken from their results, shows a wide range of variation both in the absolute bromine amount and in its relation to the corresponding chloride content.

	mg. Br/100 g. dry weight	g. Cl/100 g. dry weight	Br/Cl
Bread	0.09-0.61	0.82-1.8	0.0007-0.000046
Potatoes	0.27-1.43	0.63-0.319	0.0004-0.004
Lentils	1.02	0.012	0.083
Melon	9.45-26.2	0.765-1.04	0.012-0.025

Cooking salt contains small amounts and the present author has found a sample of such salt to contain 1 mg. Br/g. Cl, or Br/Cl = 0.001. It is thus easy to understand why the normal human blood bromine varies from one individual to another when the bromine and chlorine intakes may be widely different.

A series of bromine determinations were next done on the blood of manic depressive psychotics, untreated with bromide or iodide for at least six months previously.

Lark.	1.716 mg./100 ml.	Lo.	0.453 mg./100 ml.
Est.	0.328 "	Sh.	0.78 "
Ed.	0.512 "	Si.	0.90 "
Pr.	0.572 "	M.	0.59 "
Ant.	1.64 "	McD.	0.46 "
Ps.	0.28 "	W.	0.97 "

It is clear that these individuals exhibit the same type of variability in their blood bromine as do normal persons.

Bromine determinations were then carried out on human and animal hypophyses to find out if these glands really contained high amounts of bromine (15-30 mg./100 g. wet weight) as stated by Zondek *et al.* The method previously described for blood was used except that the tissues were desiccated so that they could be stored before the analyses without fear of decomposition. Amounts of 1-2 g. dry tissue were used for the determinations as preliminary experiments on ox hypophyses and showed only a trace of bromine. One gram of dry human hypophysis represents 5-6 g. wet tissue equal to approximately 10 human hypophyses (average wet weight of each hypophysis 0.5 g.). A preliminary alkaline hydrolysis for 4 hours on the water-bath was employed before evaporation and ashing as in the blood determinations. The human hypophyses, large numbers of which were obtained from mental hospitals, were divided into groups according to type of mental disorder. No division into groups according to age or sex was made. Table I shows that considerable variation in the bromine contents of different batches of pituitaries occurs. Unless otherwise stated, the pituitaries were obtained from patients who had had no bromide during the last six months of life. The only ones which showed excessive amounts of bromine comparable with the values of Zondek were those from mental hospital patients who had definitely received bromide during life.

Table I. *Bromine determinations on batches of hypophyses.*

		mg. Br/100 g. dry weight	mg. Br/100 g. wet weight calculated
Ox		2.8	0.6
Pig		1.35	0.27
Human:			
	(1) G.P.I.	8.27	1.65
	(2) Idiots	2.10	0.42
	(3) Miscellaneous insane	6.97	1.39
	(4) Normals	4.22	0.84
	(5) Normals	11.85	2.39
	(6) Miscellaneous insane (known to have received bromide)	46.5	9.84
	(7) " " "	133	26.6
	(8) Seniles	3.53	0.7
	(9) "	6.77	1.35
	(10) Melancholics	8.46	1.69
	(11) Manics	9.1	1.8
	(12) Miscellaneous insane (some possibility of bromide dosage)	27.9	4.7
	(13)	26.1	4.4

These results show then that the amount of bromine normally in the pituitary, which is of the same order as that in blood, is inconsistent with the belief that a bromine-containing substance is either stored or secreted by that organ.

It was then decided to investigate the relationship between bromine and chlorine in the animal body and as it was thought that *post mortem* human material was not reliable, pigs, which were available, were chosen. The organs of two pigs were examined with respect to their bromine and chlorine contents. The method of Dixon [1934] was employed for the bromine estimations, using 10–15 g. tissue, and the method of Eisenman [1929] for the chlorine estimations, using about 2 g. fresh tissue.

The results are shown in Table II.

Table II. *Results of bromine and chlorine determinations, in mg. per 100 g. tissue, on pig's organs.*

Organ	Boar			Gilt		
	Bromine	Chlorine	Br/Cl	Bromine	Chlorine	Br/Cl
Serum	1.25	314	0.004	0.75	320	0.0023
Kidney	0.445	203	0.0022	0.361	197	0.0018
Lung	0.551	218	0.0025	0.397	216	0.0018
Ovaries	—	—	—	0.647	—	—
Testes	0.334	184	0.0018	—	—	—
Liver	0.295	104	0.0028	0.213	101	0.002
Brain	0.192	126	0.0016	0.191	142	0.0014
Pancreas	0.259	110	0.0025	0.265	109	0.0025
Adrenal	0.368	107	0.0035	—	—	—
Pituitary	0.270	145	0.0019	—	—	—

The pituitary figures, which represent duplicate determinations on groups of twenty pooled organs obtained from pigs in the same sties, are clearly not very different from those found for other organs. These two pigs had been fed for 5 months previous to death on a diet, the essential constituents of which, from the standpoint of bromine and chlorine content, are given below. A rough estimate of the relative bromine and chlorine content of this diet was made, using the

results of von Damiens and Blaignan [1931] and those of the present author on cooking salt.

Constituent	Barley meal	Wheat middlings	Salt	Total
% in diet	60	35	0.33	
Cl g./100 g.	0.050*	0.06*	60.7†	
Cl g./100 g. diet	0.03	0.021	0.20	0.251 (g.)
Br mg./100 g.	0.45*	0.18*	60.7†	
Br mg./100 g. diet	0.27	0.063	0.20	0.533 (mg.)

* Data of von Damiens and Blaignan [1931].

† Data of the author.

From this table it will be seen that the Br/Cl ratio ($0.533/0.251 \times 1000 = 0.002$), although only very approximate, is roughly the same as that found in the pig tissues.

CONCLUSIONS.

Determination of the bromine distribution in the animal body leaves its rôle in the organism as mysterious as ever. It seems probable that its presence is fortuitous and dependent on food intake. No evidence has been obtained in support of the theory that a bromine-containing hormone exists in the pituitary. Bromine metabolism does not appear to bear any relation to mental disease.

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