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The bromine content of human tissue

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1. A comparison of human tissue organo-bromine residues has been made from specimens obtained in the UK, West Germany and Holland.

2. These findings, in conjunction with previous animal studies and with the geographical differences in the use of brominated vegetable oils as food additives, suggest that the high bromine levels found in the fat of tissues from UK children are due to the use of these compounds.

This study was undertaken as a result of the findings of I. F. Gaunt, P. Grasso & S. D. Gangolli (1971) and I. F. Gaunt, S. D. Gangolli & R. F. Crampton (1971). Brominated vegetable oils, which have been used as food additives for over 20 years in the UK and elsewhere, were found to produce high bromine residues in all organ fat of rats and pigs. To confirm these findings in man by direct experimentation would have created a number of difficulties, including the necessity of serial biopsies. Advantage was therefore taken of the fact that in Holland and West Germany brominated vegetable oils have not been used as food additives (for reasons unrelated to their safety in use). A comparison has been made between the bromine levels of human tissues obtained in the UK, in Holland and West Germany.

Although human tissue levels of inorganic bromine have been reported (Spector, 1956), no comparable data exist for organo-bromine levels.

EXPERIMENTAL

Human tissues. Specimens of human tissues obtained at autopsy, preserved by deep-freezing, were received together with particulars of the subject from pathologists in the United Kingdom, West Germany and Holland. Details of the specimens examined are shown in Table 1.

Determination of lipid-bound bromine. The specimen was thawed and a weighed amount of minced tissue was mixed with acid-washed sand in a steel dish. The dish and contents were dried in an air oven at 100–105° for 4 h. The dried material was transferred to a Soxhlet extractor and extracted with diethyl ether (AR) for 8 h. The extract was evaporated to dryness and the weight of fat determined. To a weighed portion of extracted fat in a nickel crucible, 2 ml of 5 N-alcoholic KOH were added; the mixture was heated on a boiling water-bath until free from alcohol, and then transferred to a muffle furnace at 500° for 2 h. The cooled ash was repeatedly extracted with hot distilled

water, made up to 10 ml, centrifuged at 3000 rev./min for 5 min and the bromine content of the aqueous supernatant fraction determined by the method of Hunter (1955).

Table 1. *Description and source of specimens examined*

Nature of specimen	Age range (years)	From UK	From West Germany	From Holland
Adipose tissue	0– 1	5	5	2
	1–11	14	16	—
	11–20	—	—	2
	Over 30	21	25	54
Liver	0– 1	—	2	—
	1– 9	1	17	—
	Over 30	21	25	1
Spleen	0– 1	—	2	—
	1–10	1	14	—
	Over 30	21	19	—
Brain	0– 1	—	3	—
	1–10	1	8	—
	Over 30	21	25	—
Kidneys	0– 1	—	2	—
	1– 9	1	14	—
	Over 30	21	10	—
Total no. of samples examined		128	187	59

RESULTS

The lipid-bound bromine contents of adipose tissue and organs from specimens collected in the UK are shown in Table 2. The samples from neonates contained no detectable amounts of bromine. The bromine levels in adipose tissue from infants were negligible, but in the specimens from children up to the age of 11, appreciable amounts of bromine were found (1·7–10·9 mg/100 g fat). The lipid-bound bromine contents in the organs from children were considerably higher than in adipose tissues. In adults, the bromine levels in adipose tissue were low and ranged from less than 0·01 to 0·43 mg/100 g fat; the values were slightly higher in the organs. There were no differences related to sex or age distribution in the values derived from the adult group.

The lipid-bound bromine levels in adipose tissues and organs of samples of West German origin are shown in Table 3. In all instances the bromine content of the fatty tissues was low, and ranged from 0·07 to 0·94 mg/100 g fat in children and less than 0·01–0·09 mg/100 g fat in adults. The lipid bromine contents of the organs were also low, ranging from less than 0·01 to 0·94 mg/100 g fat.

The results for the bromine contents in the specimens received from Holland are shown in Table 4. The results show that in all the tissues examined the bromine content was low and ranged from 0·01 to 0·25 mg/100 g fat.

The largest amounts of lipid-bound bromine were found in the tissues from children in the UK. The levels of bromine in the adipose tissue of this group were approximately ten times the amounts found in the specimens from children of a similar age-group from West Germany, and the lipid-bound bromine levels in the organs of UK origin were twenty to fifty times higher than those in the West German specimens.

Table 2. *Lipid-bound bromine contents of specimens from the UK*

Source of specimens	Age	Sex	Lipid-bound bromine content, expressed as mg/100 g fat: Adipose tissue	Liver	Spleen	Brain	Kidneys
Placenta			< 0·01 (4)				
Children	6 d	♀	< 0·01	—	—	—	—
	2 years	♂	4·23	—	—	—	—
	3 years	♀	5·15	25·2	—	—	31·7
	4·5 years	♂	4·27	—	—	—	—
	5 years	♂	3·63	—	—	—	—
	5 years	♂	2·32	—	—	—	—
	6 years	♀	2·39	—	—	—	—
	7 years	♂	3·74	—	—	—	—
	7 years	♂	4·54	—	—	—	—
	8 years	♂	5·40	—	—	—	—
	9 years	♂	10·9	—	—	—	—
	10 years	♂	4·25	—	47·4	36·4	—
	10 years	♀	6·41	—	—	—	—
	11 years	♂	1·77	—	—	—	—
	Unknown	♂	1·87	—	—	—	—
Adults	30–71 years		0–0·43 (21)	0–0·73 (21)	0–0·47 (21)	0–0·79 (21)	0–0·57 (21)

Figures in parentheses refer to number of samples examined.

Table 3. *Lipid-bound bromine contents of specimens from West Germany*

Source of specimens	Age	Sex	Lipid-bound bromine content, expressed as mg/100 g fat: Adipose tissue	Liver	Spleen	Brain	Kidneys
Children	5 d	Unknown	0·40	0·11	0·26	0·06	0·13
	7 d	♂	0·64	—	—	—	—
	3 months	Unknown	0·94	—	—	—	—
	4·5 months	♂	0·20	—	—	—	—
	4·5 years	♂	0·15	0·64	0·59	0·16	0·86
	1·5 years	♂	0·28	0·59	0·44	0·11	0·70
	3 years	Unknown	0·34	0·85	0·01	0·27	0·10
	5 years	Unknown	0·56	0·74	0·82	0·14	0·44
	7 years	♂	0·24	0·94	0·12	—	0·26
	7 years	♂	—	< 0·01	0·36	—	0·42
	8 years	♂	0·17	0·32	0·71	—	0·25
	9 years	Unknown	0·57	0·17	0·11	—	0·50
	9 years	♂	0·15	0·27	0·68	—	—
	Unknown	♂	0·07	0·35	0·17	0·47	0·31
	Unknown	♂	0·52	0·20	0·66	0·17	0·11
	Unknown	♂	0·31	0·23	0·07	—	—
	Unknown	♂	0·16	0·81	0·76	—	0·19
	Unknown	♀	0·69	0·17	—	—	0·16
	Unknown	♀	0·12	0·40	—	—	0·60
	Unknown	♀	0·82	0·43	0·16	0·71	0·85
	Unknown	♀	0·44	0·87	0·13	0·50	0·49
	Unknown	Unknown	0·39	0·83	0·30	0·14	0·52
Adults	30–75 years		0–0·09 (25)	0–0·06 (25)	0–0·09 (19)	0–0·08 (25)	0–0·06 (10)

Figures in parentheses refer to number of samples examined.

A comparison of the lipid-bound bromine levels in the specimens of adipose tissue from adults in the three countries showed that almost half the specimens had less than 0·01 mg bromine in 100 g fat. The highest levels found were in the specimens from the UK, where 5·6% of the specimens had bromine levels in the range of 0·40–0·45 mg/100 g fat. In the Dutch specimens, two of the fifty-four (1·6%) had bromine levels ranging from 0·35 to 0·40 mg/100 g fat. The West German specimens had the lowest amounts of lipid-bound bromine.

Table 4. *Lipid-bound bromine contents of specimens from Holland*

Source of specimens	Age	Sex	Lipid-bound bromine content, expressed as mg/100 g fat	
			Adipose tissue	Liver
Children	3·5 months	(1) ♂	< 0·01	—
	4 months	(1) Unknown	< 0·01	—
	18 years	(1) ♂	0·12	—
Adults	20–70 years		0·1–0·37 (54)	0·21 (1)

Figures in parentheses refer to number of samples examined.

Table 5. *Environmental bromine compounds*

Inorganic bromide	Drugs
Ethylene dibromide	Fuel additive Fumigant
Methyl bromide	Fumigant
Methylene chlorobromide	Fire extinguishers
Potassium bromate	Flour additive
Tetrabromofluorescein (Eosin)	Lipsticks
Monobromoacetic acid esters (prohibited in 1950)	Food preservative

DISCUSSION

The relatively elevated levels of lipid-bound bromine found in the UK specimens, particularly in those from children, suggest that the UK population has been exposed to bromine-containing compounds to a greater extent, resulting in the accumulation of bromine in adipose tissues in amounts larger than those found in specimens from the other two countries. The possible implication, in this context, of organo-bromine compounds in the form of drugs, industrial chemicals and food additives was therefore explored.

Some environmental chemicals containing bromine are shown in Table 5. It is difficult to see how any of these compounds could be responsible for the considerable differences in the tissue residues of bromine between UK and continental children. Inorganic compounds are readily excreted and are present only in the aqueous phase of tissues (Goodman & Gilman, 1965). Only the brominated vegetable oil has the necessary distribution to explain the epidemiological differences. Moreover, the results found in UK children are those expected from previous animal findings. Industrial com-

pounds of bromine which are known to accumulate in body fat (ethylene dibromide, methyl bromide and methylene chlorobromide) are relatively rapidly converted into inorganic bromides (Abreu & Emerson, 1940; Clarke, Roworth & Holling, 1945; Gay, 1962; Miller & Haggard, 1943; Von Oettingen, 1964; Svirbely, Highman, Alford & Von Oettingen, 1947), and would have appeared predominantly in the tissues of adult males.

The monobromoacetic acid ethylene glycol ester has never been used as a food additive in the UK. Its use as such in France and Holland was banned in 1950 owing to its toxic effects (Dalgaard-Mikkelsen, Kvorning & Møller, 1955; Le Poidevin, 1965).

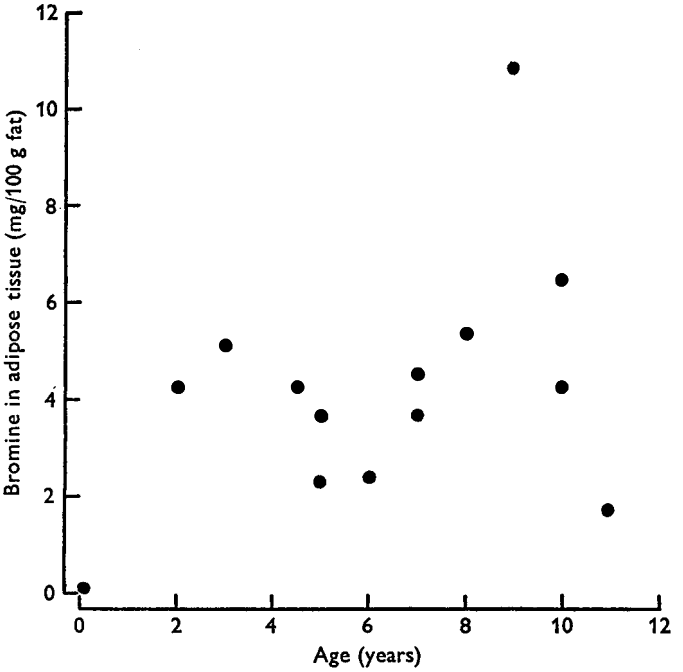

Fig. 1. Bromine concentration in adipose tissues from UK children.

In the UK, brominated vegetable oils, permitted at levels to 80 ppm in soft drinks, may contribute about 1–2 mg bromine/d, if a daily intake of about 500 ml of soft drinks is assumed. As soft drinks are predominantly consumed by children, the results presented in Fig. 1 suggest some positive correlation between age and bromine residues in adipose tissue, though more information, particularly for neonates and in the age-group of 10–18 years, is required before any more definite conclusions could be reached. Tissue from children in these age-groups is difficult to obtain. As the majority of tissue specimens were obtained *post mortem*, no information on estimated soft drink intake could be obtained. In spite of the several deficiencies in this study, it seems highly probable that the intake of brominated vegetable oil is the cause of the tissue bromine residues in children.

The use of brominated oils as food additives has been banned in the UK as from September 1970.

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