THE IRON, COPPER AND MANGANESE CONTENT OF THE HUMAN BRAIN.

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The observations recorded in this paper form part of an investigation into the mineral constituents of the central nervous system both in normal and pathological conditions.

Iron.

Iron is a respiration catalyst, and recent work has tended to emphasize the importance of this element in the brain (1). The brain cortex is very rich in cytochrome, the iron-containing pigment which plays an intimate part in cellular metabolism (2).

Exhaustive histochemical observations on iron distribution in pathological states have been made by Spatz (3), and confirmed by others (4, 5). The quantitative distribution, however, has not yet been completely studied, and there is a wide divergence in the results recorded (6–11).

The first part of this investigation consisted in the estimation of the total iron in the various parts of the brains of a large number of "accident" and pathological cases.

> The brain was washed as free from blood as possible, and portions of the cortex, white matter, cerebellar cortex and corpus striatum (putamen, nucleus lenticularis, and nucleus caudatus) were severally isolated. The dried tissue was ashed, and the iron estimated in a solution of the ash by the colorimetric method of Lyons (12). Recovery 90%. Error for duplicates $\pm$ 6·8%.

On this dry basis, the content of the cortex was the same as that of the cerebellar cortex; the white matter contained about half as much iron as the cortex; and the corpus striatum contained a large excess over that of any other part.

Of the various pathological cases, marked differences from the average occurred only in the G.P.I. cortex, many of which gave higher values. The results obtained in this way are given in Table 1.

The cases of general paralysis whose brains were submitted to the Central Laboratory for investigation are those of patients (*a*) who have died without treatment, (*b*) who have died during a course of malaria or tryparsamide treatment, (*c*) who showed no signs of remission after treatment, (*d*) whose reaction to treatment was such as to cause a complete arrest of the progress of the

TABLE I.—*Distribution of Total Iron in the Human Brain (mgrm./100 grm. dried tissue).*

		Fe.	n.	σ.
"Accident" and pathological cases:	Cortex	22·6	35	3·16
	White matter	12·6	30	2·12
	Cerebellar cortex	22·2	31	4·25
	Corpus striatum	55·5	35	13·2
G.P.I.: Cortex		29·0	19	4·62

n = number of cases estimated. σ = standard deviation.

disease, but who had to be retained in hospital on account of an irrecoverable dementia. These latter cases had died of other non-paralytic disorders at various dates up to ten years from the time of the treatment. In the majority of such cases of total arrest of the disease, histochemical examination reveals no signs of the lesions of general paralysis other than nerve-cell degeneration. All cases diagnosed as G.P.I. gave the typical serological and humoral reactions on examination before treatment.

It was found that the total cortical iron in those cases where histochemical examination revealed characteristic signs of G.P.I. in the brain was significantly higher than in those cases where there were no signs of active disease:

	Fe.	n.	σ.
No signs of active G.P.I.	26·0	6	5·11
Signs of active G.P.I.	32·3	13	4·37

The results were compared by the "t" method of Fisher (13) for comparison of two means. In this case $t = 2 \cdot 60$, which is significant.

The cases were then classified according to whether or not the histochemical examination revealed a characteristic deposit, usually assumed to be iron, in the cortex. The water content was determined (brain not washed); and the iron estimated in mgrm./100 grm. fresh tissue. It was found that those cases having a histochemical deposit were significantly higher ($t = 3 \cdot 80$) than those having no deposit:

	Fe.	n.	σ.
No deposit	3·74	10	0·56
Deposit	5·05	17	0·98

With three exceptions, in 37 G.P.I. cortexes examined histochemically and compared with the general appearance of the brain, there was no histochemical deposit when there were no characteristic histological appearances of general paralysis; and there was a deposit when there were characteristic signs of G.P.I. The exceptions were: two cases showing no deposit where there was a slight appearance of G.P.I., and one case having no appearance of G.P.I.

where a deposit was observed in the cells (or ? neuroglia) and not in the capillaries.

The next step was to ascertain whether this excess of total iron found in the G.P.I. cortex was due to an excess of blood (50 mgrm. Fe/100 ml. blood), or whether the excess was in the non-hæmatin or "available" portion.

There are several methods for estimating "inorganic" (11), "available" (14, 15), or "ionizable" (16) iron in tissues, but there is no evidence that, by any of these methods, all the non-hæmatin iron is extracted; and, in the case of the Tompsett (11) technique, this cannot be so, because I have obtained very much higher values by leaving the tissue overnight in contact with the reagents, than for 30 minutes, as advised by Tompsett, maximum values being attained in about 24 hours. In this time it is probable (as a result of experiments not detailed here) that, in the case of the cortex and corpus striatum, all the non-hæmatin iron is extracted; and in addition a very small amount of blood iron, insufficient to affect the results significantly, i.e., significant variations will not be due to blood. I have given the results obtained by the Tompsett technique, leaving the tissue in contact with the reagents for 30 minutes and 24 hours, and have referred to these respectively as representing "inorganic" and "available" iron.

Table II shows that the average "normal" distribution of total and "available" iron per 100 grm. fresh tissue is approximately equal throughout the cortex, white matter, and cerebellar cortex; with a large excess, mostly "available", in the corpus striatum, and particularly in the pallidus and substantia nigra.

TABLE II.—*Distribution of Total and "Available" Iron in the Human Brain (mgrm./100 grm. fresh tissue).*

	H_2O%.	n.	σ.	Total Fe.	n.	σ.	"Available" Fe.	n.	σ.
Cortex	83·0	18	1·78	3·62	19	0·62	2·81	7	0·63
White matter	68·5	16	2·50	3·77	17	0·48	2·59	6	0·76
Cerebellar cortex	81·5	13	1·88	3·68	14	0·49	2·83	5	0·79
Corpus striatum	79·4	23	1·72	11·50	28	2·35	9·92	8	2·14
Pallidus	73·6	4	2·15	20·60	5	3·51	22·10	4	9·00
Substantia nigra	70·8	3	2·64	20·86	3	6·50	26·80	1	
Thalamus	75·5	7	2·68	6·26	8	2·29	5·96	3	0·68

Estimation of the "available" iron in the cortex of the G.P.I. gave the following results:

	Fe.	n.	σ.
No deposit	2·69	5	0·368
Deposit	4·46	8	0·506

There is a significant (t = 5·96) excess of "available" iron in those cases where a deposit was observed histochemically.

In Table III details are given of those cases where the total and "available" iron was determined on the same sample. In the case of the G.P.Is. there was also a significant (t = 5·97) excess of "available" iron in those cases where a deposit was observed histochemically. These results show that deposits of iron demonstrated by histochemical methods are not the expression of a redistribution of the normal cellular iron content, but represent iron deposition from extracellular sources. The frontal, medial and occipital regions of the cortex were, in several cases, taken for analysis. In the case of the G.P.Is. the frontal region sometimes contained most iron; and, where the three regions were analysed, the value for the frontal region only is cited in the table. The value for the pallidus in Case 186 has been given, because this part is specifically affected in CO poisoning (5); the iron content in this case was normal.

TABLE III.—*Total and "Available" Iron in the Cortex of the G.P.I. (mgrm./100 grm. fresh tissue).*

	Case No.	Total Fe.	"Available" Fe.	
"Normal"	186	3·28	2·62	CO poisoning (pallidus 20·02).
	209	3·57	2·65	Diabetes.
	228	3·76	3·24	Accident.
G.P.I. (no deposit)	196	4·12	2·76	No appearance of G.P.I.
	202	3·78	2·47	Very slight appearance of G.P.I.
	212	4·38	2·50	No appearance of G.P.I.
	236	3·70	3·30	,, ,,
	232	3·54	2·42	,, ,,
G.P.I. (deposit)	188	5·25	3·84	Frontal region. Well-marked appearance of G.P.I.
	189	5·50	4·38	Ditto.
	194	6·11	5·33	Deposit not in capillaries. No appearance of G.P.I.
	216	4·28	4·24	Very slight appearance of G.P.I.
	224	5·91	4·45	Typical appearance of G.P.I.

Table IV shows that there is less iron in the brains of children than in those of adults, and that normal figures are reached at 11 years. The iron content of children's blood is approximately the same as that of adults (17).

TABLE IV.—*Iron in the Brains of Children.*

		H_2O%.	Total Fe.	"Available" Fe wet basis.	Total Fe dry basis.
Newborn:	Cerebrum	87·4	0·90	0·81	..
4 days:	,,	88·7	0·80	0·42	..
8 months:	Cortex	85·8	1·80	1·27	..
	Corpus striatum	79·7	2·70	..	..
	Pallidus	76·8	7·37	..	..
2 years:	Cortex	84·1	1·34	..	..
	Corpus striatum	82·4	1·83	..	..
4 ,,	Cortex	..	..	..	12·9
	Pallidus	..	..	..	23·2
6 ,,	Cortex	..	..	..	14·1
11 ,,	,,	..	..	..	18·2
	Corpus striatum	..	..	..	76·5

In Table V are given the results obtained when the tissue is in contact with the reagents for 30 minutes as advised by Tompsett (11). This iron, referred to by Tompsett as "inorganic", appears in excess in the corpus striatum, but not in those G.P.I. cortexes showing a histochemical deposit.

TABLE V.—*Distribution of "Inorganic" Iron in the Human Brain and G.P.I. Cortex (mgrm./100 grm. fresh tissue).*

	Fe.	n.	σ.
"Normal": Cortex	1·28	10	0·32
White matter	1·23	9	0·27
Cerebellar cortex	1·39	8	0·38
All cases: Corpus striatum	3·21	13	0·84
Thalamus	2·15	4	0·36
2 years: Corpus striatum	0·16	..	..
4 ,, Cortex	0·69	..	..
G.P.I. no deposit: Cortex	1·19	4	0·22
,, deposit: ,,	1·29	5	0·44

Estimation of the total iron in sciatic nerve and in the spinal cord gave the following results (mgrm./100 grm. dried tissue):

	Fe.	n.	σ.
Sciatic nerve	6·6	5	0·985
Spinal cord (tissue preserved in formol)	6·3	8	0·694

It will be seen that sciatic nerve gives a much smaller value for the total iron than the white matter of the corpus callosum. It would appear improbable that the medullated nerve-fibres of the peripheral nerve have a markedly different mineral constitution to the medullated fibres of the central nervous system. It is therefore possible that the chief iron-containing constituent of the central white matter is not the nerve-fibre but the neuroglia. Some probability is given to this view by the analysis of a homogeneous glioma removed from the brain. This showed a total iron value of 20·4 mgrm/·100 grm. dried tissue. It is true that the tumour neuroglia cell cannot be assumed to have the same constitution as the normal supporting glia, but should this be so, these results would indicate that the iron of the nerve-fibres is very much less than analysis of cerebral white matter would indicate. In the case of the sciatic nerve, the iron value again represents that of the medullated fibre together with an unknown amount of iron containing connective tissue. A separate estimation of the iron of the connective tissue of sciatic nerve gave a value of 5·6 mgrm./100 grm. dried tissue.

Copper.

In his review on the biological significance of copper, Elvehjem (18) states that this element is present in all living matter, and that a complete understanding of the action of copper will bring us distinctly nearer an understanding of the fundamental processes of living matter. Traces of copper (or iron) can render reduced glutathione autoxidizable (2).

Copper has been estimated quantitatively in the whole brain (8, 11, 19, 20), and in the cerebellum in a single instance (20), but not in the various parts of the brain.

I have estimated the copper according to Haddock and Evers (21) in a solution of the ash of the tissue (recovery 90·5%; error for duplicates ± 6·5%), and have obtained the results given in Table VI:

TABLE VI.—*Distribution of Copper in the Human Brain (mgrm./100 grm. fresh tissue).*

	Cu.	n.	σ.
Cortex	0·43	11	0·126
White matter	0·29	11	0·062
Cerebellar cortex	0·40	11	0·141
Corpus striatum	0·47	8	0·132
Thalamus	0·41	1	..
Pallidus	0·60	3	0·066
Substantia nigra	1·45	3	0·316
Cerebrum (newborn)	0·18	2	0·070
,, (1 hour)	0·18	1	..
Cortex (2 years)	0·36	1	..

The copper is presumably all "available", because Tompsett (22) found that the copper may be extracted completely from tissues by trichloracetic acid. No relative excess of copper was found in the corpus striatum, such as exists in the case of iron; but the pallidus contained more copper than the cortex; and in the substantia nigra a large excess of copper was found: in this case it was necessary to combine the tissue from a large number of brains, and three such combined estimations were made. As the substantia nigra contains large amounts of melanin, the excess copper may have some relation to the catalytic influence of copper on the oxidation of 1–3: dihydroxyphenyl-alanine ("dopa") to melanin (19). The ink-sac of the octopus, the ink of which contains melanin, has a very large copper content (19). The substantia nigra of the ox brain, which does not contain melanin, has about half as much copper as the substantia nigra of the human brain. The copper content of the cortex was normal at 2 years.

The distribution of copper in the ox brain is given in Table VII for comparison with human brain. The ox cortex and cerebellar cortex contained nearly twice as much copper as the corresponding parts of the human brain. [The ox liver, particularly in the newborn, contains very much more copper than that of the human liver (19). The copper content of ox blood is not higher than that of the adult (23).] The substantia nigra from ten brains gave enough tissue for a single estimation.

TABLE VII.—*Distribution of Copper in the Ox Brain* (*mgrm.*/100 *grm. fresh tissue*).

	Cu.	n.	σ.
Cortex	0·77	6	0·171
White matter	0·23	5	0·182
Cerebellar cortex	0·77	4	0·292
Corpus striatum	0·35	5	0·123
Substantia nigra	0·75	1	..

Manganese.

Manganese is a regular constituent of animal tissues, in which it has important metabolic functions (24). Animals treated with manganese compounds show degenerative changes in the central nervous system (24).

This element has been estimated in the human brain by Reiman and Minot (25), who obtained values of 0·029, 0·024, 0·032 mgrm./100 grm. fresh tissue for a baby, a child of 4 years, and an adult (anæmia) respectively.

Estimations of the manganese content in the various parts of the human brain gave the results set out in Table VIII:

TABLE VIII.—*Distribution of Manganese in the Human Brain* (*mgrm.*/100 *grm. fresh tissue*).

		Mu.	n.	σ.
Adults:	Cortex	0·019	13	0·0052
	White matter	0·031	13	0·0106
	Cerebellar cortex	0·023	6	0·0029
	Corpus striatum	0·053	3	0·0025
Newborn:	Cerebrum	0·020	1	..
1 hour:	,,	0·025	1	..
2 years:	,,	0·017	1	..

Owing to the comparatively low content, it was necessary to take at least 8 grm. dried tissue for each estimation. For this reason only certain portions of the nervous system could be examined. In the case of the corpus striatum it was necessary to combine the tissue from several brains. Three such combined estimations were made.

The method employed was that of Willard and Greathouse (26), as applied to biological material by Skinner and Peterson (27). Recovery, 103%. Error for duplicates, $\pm$ 7·2%.

It will be seen from the table that the corpus striatum contains more manganese than any other part; and that the content of the brain at birth, 1 hour, and at 2 years, is not much less than that of the adult.

Summary.

(1) Figures are given for the distribution of iron (total, "available", and "inorganic"), copper, and manganese in the various parts of the human brain.

(2) Iron (all forms) and copper are, on a wet basis, approximately evenly distributed throughout the cortex, white matter, and cerebellar cortex.

(3) A large excess of iron (all forms) and manganese, but not of copper, was noted in the corpus striatum.

(4) The pallidus and substantia nigra were found to have the highest iron content (total and "available").

(5) A large excess of copper was found in the substantia nigra.

(6) In the G.P.I. cortex, cases in which a deposit of iron was observed histochemically gave values for total and "available" iron that were significantly higher than values obtained for cases in which no deposit was observed.

(7) The typical appearances of G.P.I. were found to be associated with the histochemical deposit of iron, and so also with the high figures for total and "available" iron.

(8) Less iron (all forms) was found in the brains of children of six years and under than in those of adults (normal at 11 years). At two years copper and manganese were approximately normal. [In the livers of children, iron, copper and manganese fall during nursing and increase during childhood (28).]

(9) In the newborn, iron and copper are low and manganese normal.

(10) The ox cerebral and cerebellar cortex were found to contain more copper than the corresponding parts of the human brain.

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Bibliography.—(1) Gerard, R. W., *Physiol. Rev.*, 1932, xii, p. 469.—(2) Holmes, E., *The Metabolism of Living Tissues*, 1937.—(3) Spatz, H., *Zeits. f. d. ges. Neur. u. Psychiat.*, 1922, lxxvii, p. 261.—(4) Marinesco, G., and Draganesco, *Compt. Rend. Soc. Biol.*, 1923, lxxxviii, p. 898.—(5) Gans, A., *Brain*, 1923, xlvi, p. 128.—(6) Freeman, W., *Arch. Neur. and Psychiat.*, 1930, xxiv, p. 300.—(7) Sheldon, J. H., *Quart. Journ. Med.*, 1927, xxi, p. 123.—(8) Ramage, H., and Sheldon, *ibid.*, 1935, xxvii, p. 121.—(9) Wuth, J., *Zeits. f. d. ges. Neur. u. Psychiat.*, 1923, lxxxiv, p. 474.—(10) Weil, A., *Zeits. f. Physiol. Chem.*, 1914, lxxxix, p. 349.—(11) Tompsett, S. L., *Biochem. Journ.*, 1935, xxix, p. 480.—(12) Lyons, E., *Journ. Amer. Chem. Soc.*, 1927, xlix, p. 1916.—(13) Fisher, R. A., *Statistical Methods for Research Workers*, 1930, p. 107.—(14) Hill, R., *Proc. Roy. Soc.*, B, 1931, cvii, p. 205.—(15) Kohler, G. O., *Journ. Biol. Chem.*, 1936, cxiii, p. 49.—(16) Shackleton, L., and McCance, *Biochem. Journ.*, 1936, xxx, p. 582.—(17) Sobel, I. P., and Drekter, *Amer. Journ. Dis. Child.*, 1933, xlv, p. 486.—(18) Elvehjem, C. A., *Journ. Biol. Chem.*, 1935, xv, p. 471.—(19) Cunningham, I. J., *Biochem. Journ.*, 1931, xxv, p. 1267.—(20) Bodansky, M., *Journ. Biol. Chem.*, 1921, xlviii, p. 361.—(21) Haddock, L. A., and Evers, *Analyst*, 1932, lvii, p. 495.—(22) Tompsett, S. L., *Biochem. Journ.*, 1934, xxviii, p. 1544.—(23) McFarlane, W. D., *ibid.*, 1932, xxvi, p. 1022.—(24) von Oettingen, *Physiol. Rev.*, 1935, xxv, p. 175.—(25) Reiman, C. K., and Minot, *Journ. Biol. Chem.*, 1920, xlii, p. 329.—(26) Willard, H. H., and Greathouse, *Journ. Amer. Chem. Soc.*, 1917, xxxvii, p. 2366.—(27) Skinner, J. T., and Peterson, *Journ. Biol. Chem.*, 1930, lxxxviii, p. 347.—(28) Ramage, H., *et al.*, *Proc. Roy. Soc.*, B, 1933, cxiii, p. 308.