# THE IRON CONTENT OF THE HUMAN BRAIN.—II.*

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## Introduction.

In a previous paper (1) the iron, copper and manganese content of the human brain were recorded, with special reference to the G.P.I. cortex, which in certain cases contained an excess of both total and "available" (i.e., non-hæmatin) iron.

The object of this paper is to give the results of further and confirmatory work on the same lines; and to record the results of work done in an endeavour to ascertain more exactly with what chemical constituents the iron of the G.P.I. cortex and other parts of the brain is associated.

## Various Forms of Tissue Iron and Their Estimation.

Tissue iron may be conveniently classified thus:

- (A) Non-hæmatin
  - (1) Non-protein
    - (*a*) Water soluble, alcohol-ether insoluble (including inorganic phosphate and sulphate).
    - (*b*) Alcohol-ether soluble (mostly lipoid).
  - (2) Protein.
- (B) Hæmatin.

The estimation of (A) may be effected for the cortex, cerebellar cortex and corpus striatum, as described in the previous paper (1), by the Tompsett technique (2), modified by leaving the tissue in contact with the reagents for twenty-four hours. In this way all the non-hæmatin iron is extracted and the hæmatin iron is not significantly extracted. No more iron is liberated when the extraction is effected at 37° C. than at room temperature. Sodium pyrophosphate, the alternative reagent specified by Tompsett (2), should be used in place of the thiolacetic acid reducing agent, because the latter extracts hæmoglobin iron (3).

The estimation of the non-protein iron can be carried out by successively

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extracting the fats and water-soluble constituents with boiling alcohol, ether and water (4).

The estimation of the hæmatin iron may be effected colorimetrically as acid hæmatin by extracting the tissue with a solution of 5% HCl in acetone. Average figures for iron due to blood in mgrm./100 grm. fresh tissue are: Normal cortex, 0·68; G.P.I. cortex, 0·77; cerebellar cortex, 0·95; corpus striatum, 0·93. These figures multiplied by two give the ml. blood/100 grm. fresh tissue.

The techniques of Shackleton and McCance, and Kohler (5, 6) give less than all the non-hæmatin iron. This is shown by the fact that the iron rendered available increases with the time, temperature, and H-ion concentration, factors of the extracting fluid (Table I).

TABLE I.—*Increased Fe Rendered Available by Varying Conditions of Time, Temperature, and H-ion Concentration. (Dipyridyl with Hydroquinone Reducing Agent.) Expressed as mgrm. Fe/100 grm. Fresh Tissue.*

| Time. | Temperature. | pH. | Pallidus. | Corpus striatum. |
|---|---|---|---|---|
| 48 hours | Room | 5·5 | 3·25 | 1·61 |
| 48 ,, | ,, | 3·0 | 9·00 | 6·46 |
| 10 days | ,, | 3·0 | 20·04 | — |
| 48 hours | 37° C. | 3·0 | 20·50 | 12·02 |

That this increase is not hæmatin iron is proved by the fact that the $\alpha$–$\acute{\alpha}$ dipyridyl reagent of Hill (7) employed will not react with reduced hæmatin. These techniques, even that carried out at pH 5·5, give no exact measure of non-protein iron, or of inorganic iron, becase they cause the liberation of protein iron. It was found that the iron in the protein residue after alcohol-ether-water extraction may be rendered progressively available by decreasing the pH or increasing the temperature.

Values obtained for "available" iron at pH 5·5 (5), pH 3·0 (6), and pH 3·0 at 37° C., which are not detailed here, show a progressive increase, until at 37° C. values approximating to those for non-hæmatin iron by the modified Tompsett technique are obtained. Increased G.P.I. cortex iron is shown by the two last-mentioned techniques, but not by the first. If this increase is in the water-soluble portion, it might be expected to show by the first-mentioned technique and also by that employed for "inorganic" iron in the previous paper (1). That it does not do so is probably due to the fact, already stressed above, that protein iron is also liberated, i.e., non-protein, lipoid and protein iron are liberated incompletely and together.

The so-called "normal" brains were obtained from cases of accidental death and from persons dying of non-nervous complaints.

The brains from cases of general paralysis were always examined for histo-chemical evidences of excessive iron. The cases failing to show a positive iron reaction were invariably found to have undergone a course of malarial treatment. Those showing a positive reaction were either untreated cases, or cases dying during or shortly after a malarial course.

The chemical estimation of the non-hæmatin iron in cases of general paralysis yields values closely corresponding to the results of the histo-chemical iron reaction. Patients who have not undergone treatment show the histo-chemical reaction for iron and have a marked increase of non-hæmatin iron. Those who have been treated show no histo-chemical reaction and the non-hæmatin iron is within normal limits. Table II gives the values for non-hæmatin iron obtained by subtracting the blood iron from the total iron.

TABLE II.—*Excess Non-hæmatin Fe in G.P.I. Cortex (Histo-chemical Fe Deposit) Shown by Estimation of Total and Blood Fe. Expressed as mgrm. Fe/100 grm. Fresh Tissue.*

| Case number. | | Total Fe. | Total Fe less blood Fe. |
|---|---|---|---|
| 247 | Normal cortex | 3·25 | 2·72 |
| 250 | ,, ,, | 2·94 | 2·37 |
| 251 | ,, ,, | 4·20 | 3·28 |
| 280 | ,, ,, | 4·05 | 3·42 |
| 232 | G.P.I. cortex (no histo-chemical Fe deposit) | 3·54 | 2·36 |
| 236 | | 3·70 | 2·86 |
| 242 | | 3·06 | 2·38 |
| 237 | G.P.I. cortex (histo-chemical Fe deposit) | 5·75 | 4·91 |
| 244 | | 4·83 | 4·05 |
| 248 | | 4·57 | 4·09 |

Direct estimations of the non-hæmatin iron confirm these values. Thus, in a series of normal brains the cortex values were 2·68, 3·06, 2·13 mgrm. %, whereas in G.P.I. brains, showing a histo-chemical iron reaction, the cortex values were 4·78, 4·28, 4·24, 4·50 mgrm. non-hæmatin iron per 100 grm. fresh tissue.

The non-protein iron is largely increased in those cases of general paralysis having a positive histo-chemical reaction and an increase of the total iron.

Table III shows the ratio of the two non-protein iron fractions both in normal cortex and that of general paralytics.

When the partition of iron in nervous structures other than the cortex is studied, it will be seen (Table IV) that the great excess over other structures found in the lenticular nucleus is chiefly attributable to the protein iron. There is a similar excess of protein iron in the globus pallidus over that found

TABLE III.—*Forms of Fe in Brain (Average Figures). Expressed as mgrm. Fe/100 grm. Fresh Tissue Originally Taken for Analysis.*

| | Total Fe. | Protein Fe. | Blood Fe. | Fe extracted by alcohol-ether-water (by difference). | Water-soluble, alcohol-ether insoluble Fe. | |
|---|---|---|---|---|---|---|
| Normal cortex (12 cases) | 3·85 | 2·52 | 0·88 | 1·28 | 1·07 | — |
| G.P.I. cortex (4 cases) | 4·45 | 2·95 | 0·88 | 1·49 | 1·30 | No histo-chemical Fe deposit. |
| G.P.I. cortex (9 cases) | 5·20 | 2·89 | 0·99 | 2·32 | 2·48 | Histo-chemical Fe deposit. |

TABLE IV.—*Average Fe Values for Normal Brain Tissue other than Cortex. Expressed as mgrm. Fe/100 grm. Fresh Tissue.*

| | Total Fe. | Protein Fe. | Blood Fe. | Alcohol-ether soluble Fe. | Water soluble, alcohol-ether insoluble Fe. |
|---|---|---|---|---|---|
| White matter | 3·85 | 2·70 | — | 1·07 | 0·41 |
| Cerebellar cortex | 4·62 | 3·35 | 1·67 | 1·50 | 0·72 |
| Lenticular nucleus | 12·25 | 7·76 | 0·94 | 4·49 | 3·16 |
| Globus pallidus | 22·13 | 13·75 | — | 8·38 | 3·55 |

in the cortex, but in the pallidus there is also a marked excess of the alcohol-ether soluble portion. No significant differences were found in the iron values obtained in these structures between normals and cases of general paralysis.

## SUMMARY.

(1) The content of various forms of iron in different parts of the human brain is recorded, including water-soluble alcohol-ether insoluble iron, alcohol-ether soluble iron, iron combined with protein and iron due to blood.

(2) Further results confirmatory to the previous paper (1) are given, showing that there is an excess of "available" or non-hæmatin iron in those G.P.I. cortexes having a histo-chemical deposit of iron.

(3) The results indicate that the excess referred to is in the non-protein iron fraction.

(4) The large excess of total iron in the pallidus and corpus striatum is, in the latter case, due largely to protein iron, but in the former case also to the iron in the alcohol-ether extract.

(5) The cortex and white matter each contain about 70% protein iron, although the percentage of protein is 47 and 27 respectively (4).

(6) The water-soluble alcohol-ether insoluble iron constitutes about 12% of the iron in the white matter, and 25% of the iron in the cortex, corpus striatum and pallidus.

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