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A PHOTOMETRICAL METHOD FOR TESTING THE PRESENCE OF IRON IN THE CENTRAL NERVOUS SYSTEM

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The relatively high iron content of the central nervous system which can be histochemically determined was already the subject of study of investigators at the beginning of the twenties. In 1922 *Spatz* (1) published a detailed survey of this question. Employing the Turnbull-blue and prussian-blue reactions he ranged the different brain centres according to the strength of the iron reaction. He obtained the strongest colour reaction in the globus pallidus and the substantia nigra. The red nucleus and the dentate nucleus were ranged into the second group, the cerebral cortex and cerebellar cortex into the first half of the third group, and the tegmentum pontis and medulla oblongata into the second part of the same group. He regards the spinal cord as apertaining to the fourth group in which he did not obtain any iron reaction at all. The centres were examined macroscopically, as well as microscopically. *Lubarsch* (2), *Metz* (3), *Müller* (4), *Struwe* (5) and others examined by similar methods the iron content of the central nervous system in normal and pathologic brains.

The grouping was also partly controlled by *Wuth* (6) by chemical qualitative examinations, he established by the Neuman reaction the iron content in 100 gr of dry substance This method yielded numerical data. The ratios approximatively agreed with Spatz's grouping, however, owing to technical reasons in the case of smaller centres, this estimation is not appliable. The same defect adhers to the hitherto used colorimetrical, photometrical and titrimetrical methods yielding numerical data.

The disadvantage of the histochemical procedure is that it only indicates the ionized iron; it is known on the other hand, that a great part of the iron occurs in organic bonds. The catalysts playing a role in cell respiration such as cytochromoxydase and other haemin catalysts contain above all such iron. The iron occuring in the form of an organic bond splits off during the post mortem autolytic processes, but also in no small extent owing to histochemical procedures. Theoretically it might be expected that in the areas containing more organic iron, a greater amount of ionized iron can be detected by histochemical procedures.

We desired to supplement by quantitatively determinating methods the histochemical method introduced by Spatz. The essential quality of our method is the photometrical measuring of the intensity of the Turnbull-reaction employed by Spatz. Therefore, even if the iron content was also not determined by purely qualitative methods, nevertheless, the photometrical data afforded a far more exact base regarding the quantity of the iron content of the different centres, resp. tissue constituents, than the methods based on purely subjective estimation had done.

Method :

Ten human brains taken out immediately after exitus were examined by the following method : 8 brains were fixed in formalin, 2 in alcohol. The former were frozen and the 2 latter ones embedded in paraffine, resp. in celloidine. The frozen slides were 40, the embedded ones 20 micron thick. On the sections of the brains, reactions with prussian-blue, on the rest, reactions with Turnbull-blue were carried out. Of 212 slides 100 were subsequently stained with carmalum for the microscopic examination of the cells, 96 were measured photometrically without being stained again, the remaining ones did not receive any separate treatment serving as controls for the photometrical measurings. The measurings were carried out immediately after the preparation of the slides as the colour reaction is unstable.

For the measurings which were made with the collaboration of Péter Ször Ph. D. a homogeneous light source of 590 m. micron wavelength was employed, they were performed in a photocell loaded with argon-rubidium in the Physical Institute of the University of Szeged. This wavelength was chosen because the light absorbing maximum of prussian and Turnbull-blue lies in the red ; however, our photocell was not sufficiently sensitive to red light, thus an average value had to be taken, in which both factors could be best brought into correlation. The logarithm of the deviation time of the electrometer is directly proportional to the light absorption, i. e. in our case, as we are not dealing with a solution, but with dispersed system, also to the light dispersion, and inversely proportional to the thickness of the slide. Thus

$$\frac{\log \frac{T}{To}}{d \log e} = a$$

T is the deviation time of the measured slide, To is the deviation time of the control, d the thickness, e the natural logarithm and a the light absorption in per cent. The value of the iron can be determined from the light absorption in the following manner : a solution of prussian and Turnbull-blue, the iron content of which has been determined is mixed in various dilutions with dissolved gelatine plates dried over mercury by the method described by *Fröhlich* (7). Small squares are cut out of the plates, their thickness is determined by a nonius and their light absorption similarly to that of the slides. The obtained values were plotted on a curve from which the iron concentration of the slide could be calculated.

Having measured the central nervous system at 9 different points in at least 10 cases the results shown on the following table were obtained :

The photometrically measured iron content of the central nervous system in mgr per cent

Serial of brain numbers	Substantia nigra	Globus pallidus	Dentate nucleus	Red nucleus	Cerebral cortex	Cerebellar cortex	Tegmentum pontis	Medulla oblongata	Spinal cord
1.	9	10	4,5	4,5	4	4	3,25	2,5	2
2.	10	11	4,5	5	4,25	4,25	3,5	2,75	2,25
3.	12	12	5	5,5	4,5	4,5	4	2,75	2,75
4.	12	12,5	5,5	6	5	5	4	3	2
5.	14	13	6	6	5	5,25	4,5	3,25	3,25
		13							
6.	15	14,5	6	6	5,5	5,25	4,75	3,25	3,25
		14,5							
7.	16	15	6,5	6,25	5,5	5,25	5	3,5	3,25
8.	16	16	6,5	6,5	6	5,5	5	3,5	3,25
9.	17	16,5	7	7	6,25	6,25	5,5	3,5	3,5
	17								
10.	18	18	7,5	7,25	6,5	6,5	5,5	4	3,75
	18								
Average value	**14,5**	13,85	5,9	6	5,25	5,2	4,5	3,2	3,05

From the table it can be seen that in fact the amount of iron content ranges as follows : the globus pallidus and the substantia nigra contain the greatest amount ; they are followed by the red nucleus and the dentate nucleus and these in turn by the cerebral cortex, the cerebellar cortex, the tegmentum pontis and the medulla oblongata ; the gray substance of the spinal cord contains the least. The smaller the iron content of the brain was, the more diffused was the reaction and the less was it possible to detect iron granules. Beneath the tegmentum of the pons the blue colour was not intense enough to be detected microscopically, it could only be seen on slides with an underlying white base. The assumption of the high iron content of the extrapyramidal system is also supported by the fact of the nonlethal carbon monoxyde poisonings attaching specifically the extrapyramidal system, the more so since it is known that carbon monoxyde forms a complex with the iron of the respiratory ferment displacing it in this manner from the respiration. It is striking that the most serious disturbances appear above all in those centres which contain the most respiratory ferments. In the white substance the iron reaction appears only subsequently to alcohol fixation and very feeble, this might be brought about by the alcohol washing the iron from the adjacent gray substance into the white one. The deficiency of iron reaction can be easily explained on the base of *Huszák*'s (8) observation according to which in the respiration of the white substance the catalysator contains copper, instead of iron.

In the 10 cases described above there were no pathologic changes, whilst in one case the brain of a patient who had died of progressive paralysis was examined. The data of this case varied from the others inasmuch as it was also visible to the naked eye that the reaction in the substantia nigra of the one side was weaker than that of the other side, r. s. 18 and l. s. 11 mgr per cents whilst in all the other cases, measuring both sides, always identical result, had been obtained. Besides this the cerebral cortex also exhibited an unusually high value 10 mgr per cent. The former finding is inconsistent with the opinion that in progressive paralysis only the reaction of the cerebral cortex is enhanced.

Summarising the result of the investigation it can be stated that the gray substance of the medulla oblongata and of the spinal cord also contain diffusely located iron.

Comparing our examinations to those of Spatz they show a progress inasmuch as they enable the quantitative determination of the relative iron values photometrically. We succeeded in measuring the intensity of the iron content — even if not extending into cellular conditions — but in relatively small areas of the brain. The examination did not of course indicate absolute iron values, as the iron reactions described above are only suitable to the determination of ionized iron in anorganic bonds, it can however, be assumed that from organic bonds post mortemly, or vitally, a larger amount of iron can only split off in places where there was from the very first biologically

more present. Finally it might still be alleged that the great iron content of the gray substance of the central nervous system is due to the rich blood supply. As we employed human brains for our experiments it was not possible to wash them perfectly free, but referring to *Huszák*'s (9) experiments we can state that he, using dogs bled to death and brains washed free with salt, also found more cytochromoxydase and iron in the cortex and in the central gray nuclei than elsewhere.

Our method for the examination of iron can be used for the measuring and evaluation of the intensity of any kind of histochemical colour reaction.

Summary

Spatz and others tested by Berliner-blau and Turnbull-blau the iron in the gray substance and in the nuclei of the central nervous system. They controlled the results only by an approximative comparison of the colours with the naked eye. The quantity of the iron may be tested by usual chemical and photometrical methods applied on brain extracts, but this is not the way on which exact localisation is possible. The author combining the two methods proved the iron photometrically in slides showing the iron reaction, being a known fact that the quantity of iron is proportional to light absorption. The average results were as follows: 1. substantia nigra 14,5 mgr %, 2. globus pallidus 13,8 mgr %, 3. dentate nucleus 5,9 mgr %, 4. red nucleus 6,0 mgr %, 5. cerebral cortex 5,25 mgr %, 6. cerebellar cortex 5,2 mgr %, 7. tegmentum pontis 4,5 mgr %, 8. medulla oblongata 3,2 mgr %, 9. spinal cord 3,0 mgr %.

The method can be applied generally in the measuring of the intensity of different histochemical colour reactions and of their quantitative determination.

Выявление железа в центральной нервной системе посредством фотометрии.

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Выволы

Железо находящиеся в сером веществе и ядрах центральной нервной системы было выявлено берлинским и турнбул синим по методу Шпаца и других. Результаты их были установлены лишь только приблизительно, путем справнения цветов с пустыми глазами. Хотя в обычных мозговых экстрактах посредством химического и фотометрического метода количество железа определяется точно, однако точную локализацию его установить не удается. Автор соединив преимущество обоих методов определил количество железа, которое в стоит соотношении с поглощенным светом путем фотометрии на препаратах показывающиеся железную реакцию с постоянной толщиной.

Средние цифры определения следующие:

1. Substantia nigra 14,5 mgr %.
2. Globus pallidum 13,8 mgr %.
3. Nucleus dentatus 5,9 mgr %.
4. Nucleus ruber 6,0 mgr %.
5. Кора мозга 5,25 мгр. %
6. Кора можечка 5,2 мгр %.
7. Tegmentum pontis 4,5 mgr %.
8. Продолговатый мозг 3,2 мгр %.
9. Спинной мозг 3,0 мгр %.

Изложенное выше выявление железа годно для измерения интенсивности количественного вычисления любой гистохимической цветной реакции.

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