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The histo-anatomical basis of magnetic sensitivity in birds

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Zoologia. — *The histo-anatomical basis of magnetic sensitivity in birds.* Nota di BRUNO SCHREIBER (*), presentata (**) dal Socio S. RANZI.

RIASSUNTO. — Viene confermata la presenza, segnalata da Walcott *et. al.*, nel capo dei piccioni viaggiatori, di una serie di strutture da lui identificate come magnetite, alle quali si dovrebbe attribuire la sensitività di questi animali al campo magnetico e alle sue variazioni naturali.

Vengono portate evidenze isto-topografiche di tre forme di sensori: particelle sparse, ammassi e infine di nuclei di eritrociti ricoperti dalle stesse.

The ability of some animals (birds, worms and snails) to perceive both artificial and natural changes of magnetic fields as been known for many years. Such sensitivity has been sufficiently proved by a set of researches on migrating birds' behaviour in connection with both natural [1], [2] and experimental [3, 4, 5] changes in the earth's magnetic field.

The failure of " homing " in pigeons during magnetic storms has been observed [6] while anomalies have been reported in the leaving direction of pigeons which had been brought to the leaving place closed in iron containers [7].

However the problem remains unsolved as to " how " birds could perceive magnetic changes and which organ or which anatomical structure could be considered the specific magnetic-receptor. This uncertainity sometimes led to perplexities even as to the evaluation of the behavioural findings themselves.

An outstanding contribution to the understanding of magnetic sensitivity in birds is given by Walcott, Gould and Kirschwink [8]. They give magnetometric proof of the existence of such an organ and define its features.

Also the discovery of the presence of magnetite in iron containing bacteria [9] and in bees [10, 11] supports the hypothesis of the nature and the anatomical position of a similar sensibility in birds.

The proof given by Walcott and Co. is as follows:

a) a unilateral area in which " permanent ferromagnetic " material has been shown in the head and in the neck of pigeons by means of a SQUIB MAGNETOMETER;

b) in the symmetrical half head some superparamagnetic material can be found;

c) the permanent magnetic material is located in bone structures which can be seen by the naked eye, their size being from 1/2 to 2 mm, which lie between the dura and the skull and are too closely associated with it to be separated;

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d) the mentioned areas when examinated using both light and electron microscope show " *clusters* " of electronic—opaque structures about 0.08–0.15 micron wide, which an electron probe analysis revealed to be rich in iron. Moreover, magnetic remanence measurement lead to the conclusion that iron is present as " Magnetite " in black crystals. The tissue housing this material results to be connective tissue in which many nerve fibers can be found (1).

In the paper mentioned, Walcott *et al.*, even if exhaustive in discussing the nature of ferromagnetic material found in the skull do not give greater details about the precise topographical location of the considered region or its histo-anatomical structures, also because pictures are lacking.

In a further paper Walcott himself [12] points out that the magnetic hysteresis which had already been measured was clearly confirmed through the histological staining of Fe in two distinct cranial regions: the first one, superior, in the Harderian gland between the eyes and the cranial summit: a second one, inferior, between the optic chiasma and the olfactory lobe.

The purpose of this comunication is the detailed description of the morphology of the structures found by Walcott *et al.* in [1].

Most important is the discovery of the involvement of erythrocyte nuclei in the storage process of the black ferromagnetic particles.

These nuclei, which are totally or only partially covered with the black material, allow the histotopographical finding of the supposed magnetic receptor organ.

We used 24 homing pigeons which had been extensively tested for their orientation skill. The head was sagittaly cut and the brain removed to be examined at the binocular microscope for identifying the presence of the black areas both in the right and in the left side. In two cases a serial microtome section was made of the whole half skull.

In 12 of the examined pigeons we were able to notice areas mainly in two anatomical regions: one " superior " in the fronto-parietal area very close to the eye socket; the second one, " inferior ", in the parieto-occipital region, very close to the labyrinth, at the side of the occipital hole.

After having been removed, these regions were histologically examined under the light microscope (2).

(1) As to the whole aspect of magnetite indentification, this is extensively illustrated in Walcott's and Presti–Pettingrew's papers. They describe the two main techniques which give a unquestionable answer in this sense, that is the *x*–raysdiffraction microprobe and the " remanence " curve.

(2) The histological technique we used is the following: 1) fixation in Bouin; 2) decalcification with 5 % nitric acid for five days; 3) paraffin wax embedding; 4) staining with a) hemallume Carazzi and eosin contrast, b) Mallory trichromatic, which differentiates the bone (red) from the connective tissue (blue) very well. With

We noticed that in the frontoparietal region wide areas exist which we call "criptae" of the diploe bone of the skull vault itself (Fig. 1).

These areas in turn contain two different structures: a) "lacunae" in which a thick wall of connective tissue which seems to be very like small artery sections, and *b*) wider "lacunae" with thin walls, looking like venous synusoides (Fig. 2). In the first one, among the erythrocytes, little black round or road shaped particles can be found, either sparse or clustered, looking like the ones described by Walcott *et al.* (Fig. 8).

In the synusoidal lacunae the erythrocytes appear more or less numerous together with large opaque-black particles thereafter called by me provisionally "Black bodies" (Figs. 3, 4). Their size and shape is nearly the same as the normal erythrocytes (8 μ length) and they appear in variable number, but much fewer than the normal erythrocytes. A third component of the cryptae are more or less threadbare nervous branches around the synusoidal lacunae (Fig. 5).

Moreover, in the parieto-occipital region, a large "egg-shaped body" is clearly visible even with naked eye. (Figs. 6, 7). This body is located between the dura and the skull and is completely filled with erythrocytes at different growth stages, among which the "black-particles" can be clearly seen, both sparse or clustered in larger or smaller units.

"Black bodies" identical to those in the criptae described above can be seen tightly crowded leaning against the outward wall of this egg-shaped body (Fig. 8).

This is a very impressive phenomenon since the black edge of the egg-shaped structures can be seen even with a small enlargement.

Doubt could arise as to the chemical nature of the above described "black bodies", since near the labyrinthian zone melanin is very common. However the H_2O_2 bleaching test for melanin showed a completely negative result both for the particles and for the "black bodies".

I think this fact alone is sufficient to suppose a metal nature of these structures, presumably due to the presence of Fe as Walcott states.

Moreover the whole skull had to be treated with HNO_3 for decalcifying the bone before microtome cutting.

The fact that the black metal structures were not affected by this treatment should yield the conclusion they are made of magnetite. As a matter of fact only magnetite among the Fe-oxides is insoluble in HNO_3.

x-ray diffraction spectral analysis is necessary for the quantitative evaluation of the Fe contents but I should like to stress here the presence of what I

this staining the erythrocytes become red and therefore are clearly identifiable with the black bodies, as they are of the same size and shape.

Some slides mounted in Canada balsam *without any staining* appear to be the best to show the presence of black bodies in the remaining tissue which can observed under phase contrast.

called " Black bodies " which have not been described by Walcott and which—beyond doubt—*are erythrocyte nuclei covered with the sparse black particles in the internal mass*. Some of them are homogeneously and fully covered, others on the contrary only in part and in these cases the few particles leaning against the surface can be clearly identified as being the same as those which can be seen free in the rest of the field.

Should the analysis confirm the magnetite nature of both the particles and the clusters like those described by Walcott, *the magnetite-coated erythrocyte nuclei* described in this paper would represent a third component of magnetic material found by Walcott in the of the skull pigeon.

One could ask why only part of the erythrocytes in this region are magnetized while the others are not. A precise answer is not difficult to give now. In fact, the hypothesis can be put forth that the covered erythrocytes are actually immature erythroblasts in which free iron ions exist in the cytoplasm and on which magnetite can deposit in a specific way in the form of a ferromagnetic protein. As is well known, in erythroblast formation a stage exists which is called by Bloom and Fawcet [13] " erythroblastosis in siderocytic phase " and in which in the basophyla erythroblasts are clearly visible some cytoplasmic vacuoles containing Fe. As a matter of fact these vacuoles are stained by Prussian blue and moreover we can observe on their membrane some aspects of micropinocytosis of particles containing free iron [14].

The other two components of the magnetic stock (which, because of their insolubility in H_2O_2 and in HNO_3, must be considered magnetite) are the sparse particles " and the " clusters ".

It has to be observed that, whereas the former can be found particularly among the blood erythrocytes " in arteries " and in the " egg-shaped body " the more or less large " clusters " appear to be spread everywhere but prevail in this latter.

In the meanwhile (May 1980), a paper by Presti and Pettingrew [15] brings a further contribution to the existence of a magnetic sensitive area in birds (*Columba* and *Zenotrichia*). While confuting the work of Walcott, since they did not find any visible magnetic structure in the skull (and this fact is strange ideed), these authors describe the presence of permanent magnetic material in the neck muscle band. In this area some diffuse spots of black magnetic material appear together with black magnetic particles included in muscle fibers.

They suppose that magnetic sensitivity is due to the coupling of the magnetite with the sensorial structures of the muscle and particularly with the " muscle spindles ". They deduct therefore that the magnetic stimuli perception should be related to muscular activity itself.

This is another contribution to the finding of magnetite in bird tissues and to the idea that this material may be responsible for the behaviour of these animals under natural changes of the earth magnetic field.

Finally we must remember the great value of Walcott's finding, which opened the way to the knowledge of a new sensorial organ, the "magnetic one". I think we should propose that this histo-anatomical complex is related to the magnetic sensitivity of birds, be named "*Walcott's Organ*".

Moreover, I think that after the results of the morphological and electronic-physical analysis of the black material, further research should be directed towards electro-physiological analysis to show the possible presence of "nerve signals" for variable magnetic fields over the pigeon's head. The exact position to insert the electrode in the head could be suggested by the results of the morphological findings themselves.

To conclude, we can say that the value of such a system for the orientation ability of pigeons will be fully verified, especially by comparing the same with the corresponding structures of "non" homing pigeons.

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EXPLANATION OF PLATE I

Fig. 1. - General View of a "bone crypta" of the skull diploe bone of the frontoparietal region. In the inner area are visible the erythropoietic "lacunae".

Fig. 2. - Enlargement of part of Fig. 1 in which are present the three elements of the lacunae: arterial (a), vein (b) and nerve (c) branch.

Fig. 3. - "Black bodies" of the border of the egg-shaped region.

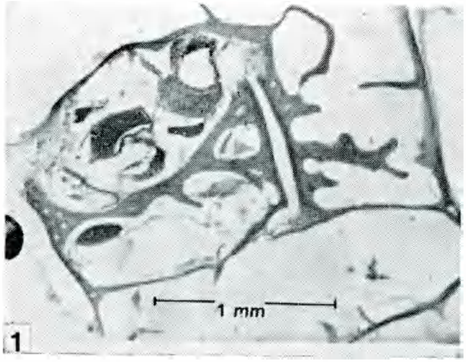
Fig. 4. - Totally (a) or partially (b) black covered erythrocytes which appear both in the spaces contained in the cryptae and in the border area of the egg-shaped body.

Fig. 5. - Nerve branch spreading over a black body region.

Fig. 6. - General view of the "egg-shaped" region (a) situation between the skull (b) and the brain (c).

Fig. 7. - Microphotography of the "egg-shaped body" located in the parieto-occipital region of the skull. Clearly visible the black border (a) consisting of few layers of the "black-bodies" which seem to be erythrocyte nuclei coated with the black particles of the supposed magnetite. The inner part of the egg-shaped body is filled with erythrocytes in different growth stages and among them are visible free or clustered black particles (see Fig. 8).

Fig. 8. - Small black-particles free (a) or in clusters (b) of the central part of the egg-shaped body and in the cryptae. It is to be noted that all the particles here described as "black" are visible in such a way also in the unstained histological slides.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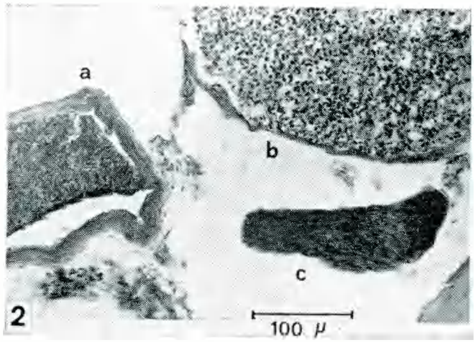
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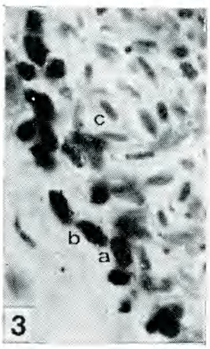
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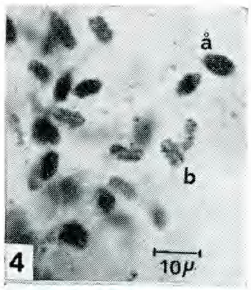
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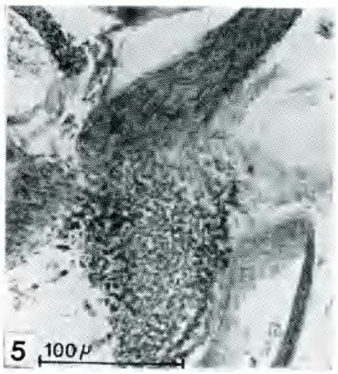
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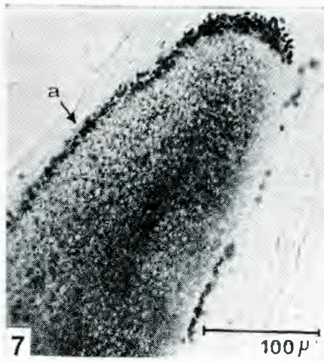
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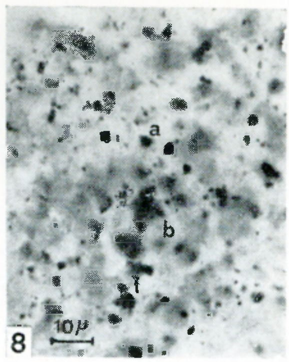
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