DEMONSTRATION OF ^{82}Br IN NERVE CELLS

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The halogen ions chlorine and bromine have long been considered to be extra-cellular tissue electrolytes. Manery and Hastings (1939) concluded from a study of the distribution of electrolytes in mammalian tissue that the central nervous system belongs to the "tissues which have a large proportion of chloride-free cells" and the current opinion is that the nerve cells of higher animals do not contain any halogen ions. Several studies show that halogen ions occur in the nerve cells of lower animals (Bear and Schmitt, 1939; Steinbach, 1941). The present study aims at showing that these ions also occur in the nerve cells of mammals.

Nerve cells from the cerebellar hemispheres of cats were studied. We chose the cerebellar cortex because we had observed that it took up more of administered bromine than the white matter (Brattgård and Lindqvist, 1954a and b). Furthermore, a gyrus from the cerebellar hemisphere contains several different kinds of tissue, a layer with many nerve cells (granular layer), a layer with few nerve cells but many dendrites (molecular layer), a layer containing many medullated neurites (nerve fibre layer) and also a layer with isolated, relatively large cells (Purkinje cells).

Method

The plan we adopted was to inject ammonium bromide containing the isotope ^{82}Br and to study how it was distributed in the cerebellar hemispheres seven hours later by means of radioautographic film sensitive to beta radiation.

The difficulty was to keep the bromine in the position it had at death. It dissolves so easily in both water and alcohol that the ordinary methods of preparing the tissue had to be modified. Mellgren (1952), with whom we collaborated, published a method for the radioautographic study of ^{82}Br in which the bromine ion is precipitated with silver nitrate. When a section is immersed in a 1% solution of silver nitrate for one minute it retains its activity no matter what is done to it afterwards. This method was used in the present study.

Six cats weighing 2.8 to 3.2 kg. were used. Each animal received an intraperitoneal injection of 0.2 g. of ammonium bromide dissolved in 2 ml. of physiological salt solution. At the time of the injection the activity of the injected solution was 3.5–4 mc. Seven hours later the animals were killed and the cerebellum removed. Cytopathological examination of different regions in the central nervous system showed no signs of radioactive injury. Small pieces of the hemispheres were fixed according to a rapid freezing-drying method (Gustavsson, 1952). After 18 hours of fixation the tissue was embedded in liquid paraffin. Three hours later it was cut into 5 or 10 μ thick sections. The sections were mounted on glass slides which had been treated so as to make photographic film fasten on them (see following). The activity of each section was estimated in a Geiger-Müller counting tube. The paraffin was removed by dropping warm ether on the slides. The sections were immersed in 1% solution of silver nitrate for one minute and then put into absolute alcohol. Some of them were put directly into absolute alcohol saturated with silver nitrate. They were tested after each treatment to check that they had not lost any activity. The radioautographic film (Kodak autographic plate) was put on water and then drawn over the sections. The film was exposed for five days in a temperature of + 4°C. in an exsiccator. The development was done in Kodak D 19b. The sections were stained through the film membrane with celestine blue haematoxylin according to the Doniach and Pelc (1950) method.

Two different tests were used to ascertain the amount of background activity in the film. The paraffin block from which the sections were taken was re-sectioned two weeks later and another film exposed under the same conditions. Bromine activity drops to one thousandth of its original value after two weeks and so any darkening of the film after that period must have come from the "non-specific" background activity. The other test was done by exposing sections not treated with silver nitrate. The bromine was dissolved with water, as was established by study of sections in a Geiger-Müller counting tube. Any darkening in plates subsequently exposed could then be assumed to be "non-specific" or background. Both tests showed only negligible darkening, diffusely spread over the whole film.

FIG. 1.—Radioautograph showing distribution of ^{82}Br in the cerebellum. The film membrane was loosened from the underlying tissue. Note the intense darkening in the convex granular layer compared with the molecular layer and nerve fibre layer. $\times 170$.

Results

The investigation showed that the ^{82}Br was unevenly distributed in different regions of the cerebellar hemispheres (Figs. 1 and 2). The film over the granular layer showed the most intense darkening. The molecular layer showed less and the least of all was seen in the nerve fibre layer. The three layers were fairly sharply demarcated from

FIG. 2.—Radioautographic film on unstained section from the cerebellum. Note the more intense darkening over the granular layer (K) than the molecular layer (M) and nerve fibre layer (N). The darkening from the pial vessels is seen uppermost in the picture. $\times 170$.

one another. In some sections there was intense darkening over the Purkinje cells, especially around their nuclei. The blood vessels produced powerful darkening in all the sections (Figs. 1 and 2). Fig. 4 shows two granular layers and an intermediate nerve fibre layer from the same gyrus. Note the large difference in the darkening over the two granular layers. On more careful focusing with higher resolution and magnification (Zeiss panchromatic microscope, objective $\times 60$, aperture 1.0, ocular $\times 10$, immersion fluid $n = 1.52$) it was possible to distinguish optically between the film layer and the stained section shown in Fig. 4. Fig. 5 shows a photograph when the darkening in the film layer was brought into focus. The darkening not only occurred over the intercellular spaces. It was also seen over regions in which the whole 5μ thick section was composed of cytoplasm. Similar darkening was seen over the nuclei. In some sections it seemed to be more intense over the cytoplasm nearest the nuclear membrane. However, this condition was not seen in all the cells.

FIG. 3.—Radioautographic film on unstained section from a gyrus in the cerebellum. Note the more intense darkening over the granular layer (K) than over the molecular layer (M). The region between these two layers shows more intense darkening over the Purkinje cells. $\times 60$.

Discussion

According to the older literature, the halogen ions chloride and bromine do not occur intracellularly (see Goodman and Gilman, 1941, and Møller, 1941, for references). This conclusion was mainly based on studies on muscular tissue (Boyle, Conway, Kane, and O'Reilly, 1940; Fenn, 1936; Gersh, 1938; Manery and Hastings, 1939). It was assumed that the same was the case in other tissues, including nerve tissue (Manery and Hastings, 1939). This made it difficult to explain the sedative effect of bromine on the cells of the central nervous system (Goodman and Gilman, 1941; Møller, 1941). In view of the results of our radioautographic study, the conclusion must be wrong as regards the nervous system of the cat.

Steinbach (1941) demonstrated the intracellular presence of chlorine in axons from the squid. Bear and Schmitt (1939) showed that the axoplasm in nerves from *Loligo* has an ionic composition differing in some respects from that of the extra-cellular fluid. According to them, the ratio between intra-cellular and extra-cellular chlorine is 1:4. Wilbrandt (1937), studying the potentials in crab nerves, said that it was obvious that the nerve membranes were not completely impermeable to anions like chlorine and bromine. Several authors (Höber, 1947; Hodgkin and Katz, 1946) have assumed that chlorine also occurs intracellularly in the central nervous system of higher animals.

FIG. 5.—Radioautographic film on cerebellum stained with celestine blue haematoxylin. The photograph shows a detail from Fig. 4, the region marked K_2 . The film layer is sharply focused and the black granules are distinctly visualized. Objective—Zeiss apochromatic $\times 60$, aperture 0.95, compensatory ocular $\times 10. \times 3,000$.

The conditions found in lower animals tally well with our observations in the nerve cells of the cat.

Summary

A radioautographic study was made of the distribution of radioactive bromine ions in different regions in the cerebellar hemispheres of cats. It was observed that the bromine content was greatest in the granular layer, less in the molecular layer, and least in the nerve fibre layer. The bromine ion occurred both inside and outside the cells and in both the nucleus and cytoplasm. The observation of halogen ions within the nerve cells of a mammal is discussed in the light of recent chemical and physiological research.

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FIG. 4.—Radioautographic film on cerebellum stained with celestine blue haematoxylin. At K_1 and K_2 are granular layers with about the same cellular density and same staining of the cellular structures. Note the large difference in the amount of shading caused by these two layers and the amount caused by them and the nerve fibre layer (N). $\times 350$.