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Radiopotassium Autography of the Rat's Brain.

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The autoradiographic technic provides an interesting and valuable approach to the histophysiology of certain radioactive isotopes in studies in detail of their intratissue distribution. Thus far the method has been applied chiefly to studies of radioactive iodine metabolism in the thyroid.[1-3] Radioiodine is concentrated in the thyroid to a much greater extent than in any other tissue,[1] whereas K^{42} is taken up very slowly and in small amount by the brain.[4] For this reason the feasibility of K^{42} autography of the brain might be questioned. Actually, however, the procedure does not offer any unusual difficulties.

The sequence of operations was as follows: weighing of the rats; intraperitoneal injection of a dose of K^{42} proportional to the weight of the animal; anesthetization, after a period allowed for assimilation of the K^{42}, and removal of the brain by craniotomy; fixation of brain slices 3 to 4 mm thick in absolute alcohol; infiltration of the tissues with 50% xylene and 50% paraffin, and then with paraffin alone; blocking, sectioning and fixing the microtome sections to glass microscope slides; exposure of a small strip of X-ray film to each slide; and development of the exposed film.

The K^{42}, as the chloride, was injected intraperitoneally in a 5.44% aqueous solution, the dose being 0.75 millicuries per 100 g of body weight. Following this, a period of 12 to 18 hours was allowed for the radioactive potassium to approach equilibrium in the brain, since this substance has been shown by Noonan, Fenn and Haege[4] to have a very slow turnover in this organ.

In this connection it was necessary also to consider the rather short half life of K^{42} (12.4 hours). It was found that if longer periods were allowed after injection, the decay of the isotope more than offset the advantage of a closer approach to equilibrium.

The dose employed was relatively large because it has been shown that only a very small amount of injected K^{42} is taken up by the brain, as compared with other tissues,[4] and because a certain minimum of radioactivity within the tissue sections is necessary to bring about adequate exposure of even the most sensitive photographic emulsions, especially when very long exposure times are precluded by a short half life of the isotope.

The fixative used was absolute alcohol, because it is necessary to exclude any water, which would affect distribution of electrolytes within the tissue. This brought about sufficient hardening of the tissue slices within 2 hours. An hour in warm xylene-paraffin, followed by an hour in paraffin alone, brought about satisfactory infiltration.

The optimal thickness for the microtome sections was found to be 6 to 12 μ. Thicker or thinner sections did not cut well. Furthermore very thin sections did not contain enough of the radioactive potassium to produce an adequate exposure of the photographic emulsion.

The ribbons of tissue sections were fixed to the microscope slides simply by slight warming to cause the paraffin of the sections to fuse to the glass. The operation also helped to bring about flattening of the sections on the slides. It was found that the autoradiographs were less distinct if the tissue sections were cleared of paraffin with xylene and were fixed to the slides by dipping in thinned collodion.

Alternate stained sections were prepared from each tissue specimen, to serve as histologic controls for the autoradiographs.

[1] Hamilton, J. G., Soley, M. K., and Eichorn, E. B., *Univ. Calif. Publ., Pharmacol.*, 1941, **1**, 339.

[2] Gorbman, Aubrey, and Evans, H. M., *Endocrinology*, 1943, **32**, 113.

[3] Leblond, C. P., Fertman, M. B., Puppel, I. D., and Curtis, G. M., *Arch. Path.*, 1946, **41**, 510.

[4] Noonan, T. R., Fenn, W. O., and Haege, Lorraine, *Am. J. Physiol.*, 1941, **132**, 474.

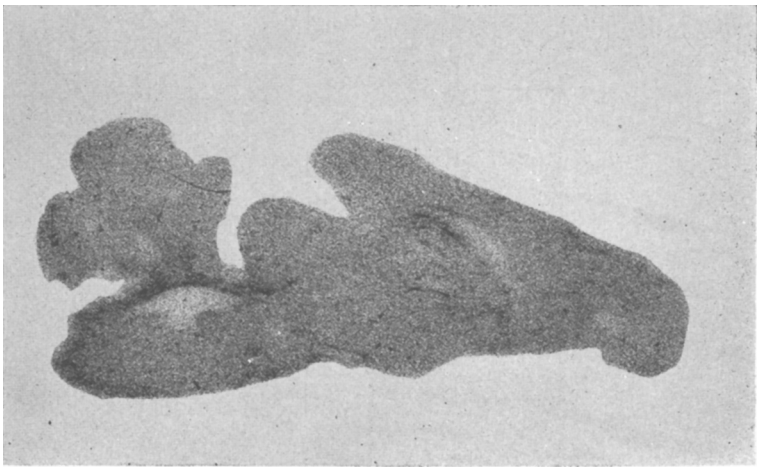

Fig. 1.
Autoradiograph showing distribution of K^{42} in a sagittal section of the brain of a normal rat 8 μ thick. The cerebral area of the autoradiograph is at the right and the cerebellar and brain-stem area at the left (× 5).

Some preparations also were made by microincinerating (according to the method of Scott[5]) sections which contained K^{42}. It was thought that removal in this way of the organic component of the tissue sections might eliminate a certain barrier between the radioactive substance and the photographic film but there was no improvement in the quality of the autoradiographs obtained by this method.

Dental X-ray films were used in the form of strips cut at a slightly greater width than that of the tissue sections. Three such strips could usually be obtained from each film. A strip of film and a tissue preparation were bound directly together, in the dark room, by wrapping them in black paper. It was important to weigh down each such film-slide preparation during the exposure period to insure intimate contact between the film emulsion and the tissue sections.

The optimal exposure time was found to range between 12 and 24 hours, depending on the thickness of the sections. If a few extra preparations are made for each experiment, films can be developed at intervals to determine when the exposure has been adequate for that particular experiment.

In a series of 16 experiments highly satisfactory autoradiographs of rats' brains were obtained by this means (Fig. 1). The normal distribution of K^{42} was found to be fairly homogeneous throughout the brain, though there frequently was the indication, by relatively greater areas of density in the autoradiographs, of somewhat greater concentrations in the cerebral and cerebellar cortex, the basal ganglia or the roof of the fourth ventricle.

Summary. The critical points of technic for the preparation of radiopotassium autographs of rats' brains have been discussed and it has been shown that good results can be obtained despite the fact that this isotope has a short half life and enters brain tissue very slowly.

It was found by this means that the distribution of K^{42} in the brains of normal rats is fairly homogeneous except for some tendency to greater concentration in the cerebral and cerebellar cortex, the basal ganglia and the roof of the fourth ventricle.

[5] Scott, G. H., *Protoplasma*, 1933, **20**, 133.