duced from 30 to 10 minutes to yield approximately equivalent values. The method has been successfully tested with over 10,000 patient blood samples. The procedure may also be used for similar proportional measurements with ascitic fluid, tissue homogenates, bacterial suspensions, etc., with no need for exact sample volume or calibrated tubes.

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1. Wintrobe, M. M., *Am. J. Med. Sci.*, 1933, v185, 58.

2. Wintrobe, M. M., and Landsberg, J. W., *ibid.*, 1935, v189, 102.

3. Cutler, J. W., *J. Lab. and Clin. Med.*, 1940, v26, 542.

4. Cutler, J. W., *Am. Rev. Tuberc.*, 1943, v48, 314.

5. Westergren, A., *Ergebn. d. inn. Med. u. Kinderh.*, 1924, v26, 577.

6. Hadley, G. G., and Larson, N. L., *Am. J. Clin. Path.*, 1953, v23, 6.

7. Queries to Editor, *J. Amer. Med. Assoc.*, 1955, v149, 1422.

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Autoradiography of Carbon-14 Labeled Isoniazid in Brain.* (22258)

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The widespread interest in drugs affecting the central nervous system, together with the increasing numbers of isotopically labeled drugs available in research, has stimulated the search for methods of producing autoradiograms of nervous tissue containing compounds labeled with such soft beta emitters as C-14. To date no suitable method has been developed for preparing brain autoradiograms for C-14 containing specimens. Previous methods have been described showing the localization of certain other isotopes not only in individual cells but even in inclusion bodies(1,2). These methods have not, however, been applied to such soft tissues as brain.

The autoradiograms described in this paper, together with supporting differential counting data, show the localization of C-14 labeled isonicotinic acid hydrazide (Isoniazid) and/or its metabolites in gross anatomical structures of brains. C-14 labeled Isoniazid was used as a prototype for these studies because of its known central nervous system effects and because it has previously been shown to localize in the nervous system of mice, rats, and guinea pigs(3). These observations on the localization of C-14 labeled Isoniazid have been confirmed by us in rats and cats, which were used in the autoradiograph studies.

Materials and methods. The Isoniazid used in these studies was synthesized by Murray and Langham of the Health Division, Los Alamos Scientific Laboratories(4). The compound was labeled in the carboxyl position and had a specific activity of 0.031 mc/mg. The labeled drug was diluted with normal Isoniazid in saline to a concentration of 2.5 mg/ml with a resultant activity of 34 x 10^6 dpm/ml. Adult cats were given an intraperitoneal dose of 10 mg/kg of Isoniazid dissolved in normal saline one hour before being anesthetized with pentobarbital sodium. During anesthesia the chest was opened and perfusion with normal saline begun in order to remove the vascular contents from the capillary bed of the brain. The left ventricle of the heart was cannulated and the descending

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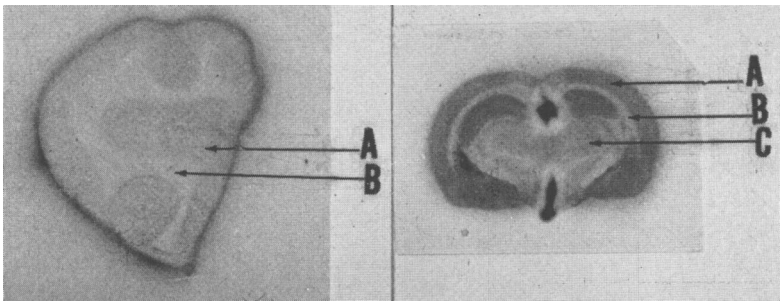

FIG. 1 (left). Autoradiogram of cat brain hemisphere showing density difference between cortex (A) and underlying white matter (B). Peripheral rim of darkest density could represent the very active spinal fluid in subarachnoid space or activity emanating from perpendicular edge of tissue block, or both. Development time, 30 days. Mylar membrane interposed between brain slice and photographic emulsion.

FIG. 2 (right). Autoradiogram of coronal section of rat brain illustrating density difference between cortex (A) and subcortical white matter (B), and showing intermediate density of thalamus (C). Development time, 30 days. No Mylar membrane used. Centrally placed, irregular dark areas represent artifact which is eliminated by the use of Mylar.

aorta was clamped. The right heart was opened to allow return of the perfusate. Two hundred ml of saline was permitted to flow through the system during a period of about 5 minutes. The perfused brains were then carefully removed and placed in a deep freeze to facilitate slicing. Serial coronal sections of approximately 3 mm thickness were made freehand with a 10-inch Valet razor blade, and one hemisphere selected for autoradiography. For C-14 assay purposes, grossly visible anatomical structures were usually selected from the opposite hemisphere, but in some cases it was necessary to obtain additional tissue from the adjacent slice in the same hemisphere. It was found impractical to separate some of the smaller structures (globus pallidus-putamen), and these are represented as single tissues in the accompanying table. Prior to assembly, the tissue slices to be used for autoradiography were placed on a glass slide and chilled to –26°C in a deep freeze, together with black plastic slide boxes (Clay Adams Co., Inc., Cat. No. A1604 B) and foam rubber inserts. The unit was assembled in the dark room by placing Kodak No Screen X-ray film on the floor of the box, covering the free surface of the tissue on the slide with a thin Mylar (du Pont) membrane, and then quickly apposing the tissue and film with the interposed membrane. In our experience the most suitable protective membrane was one quarter mil type A Mylar, which screened out 26% of the beta particles as measured in an open window gas flow Geiger counter. Firm apposition of the tissue slice to the plastic protected photographic emulsion was maintained by placing the foam rubber insert over the inverted glass slide and adherent tissue before closing the slide box. The assembled units thus prepared were sealed with black electrical tape (Minnesota Mining and Mfg. Co. No. 33), and stored at –26°C for 30 days to allow development of the autoradiogram. It is imperative that the dark room operation be carried out rapidly in order to prevent warming of the tissue with resultant softening and distortion due to the slight pressure of the foam rubber. If several autoradiograms are to be prepared at the same time, a pan of dry ice may be used in the dark room to keep the boxes chilled and the tissue slices frozen during the required manipulations. After the 30-day exposure, the film was allowed to come to room temperature before processing. The tissue slices may be fixed in neutral formalin for comparative study.

Results. It may be seen from the illustrations (Fig. 1 and 2) that there is a well defined differential density between various anatomical structures of the brain. It is significant that the relative density of the labeled Isoniazid autoradiograms in the various brain areas is similar to the differential radioactivity of the equivalent anatomical areas as

TABLE I. Activity of C-14 Labeled Isoniazid in Brain Tissues of the Cat. See also Fig. 1. Data by direct plating(6).

Tissue	dpm/mg dry wt
Cerebellar cortex	466
Cerebral "	451
Hippocampus	430
Caudate nucleus	373
Thalamus	380
Cerebral white matter	220
Globus pallidus-putamen	233
Pyramids	175
Brain stem	216
Centrum of brain stem	179
Cerebellar white matter	197
Cerebrospinal fluid	3485*

* dpm/ml.

demonstrated by gross dissection and radioassay (Table I). For example, the cerebral cortex shows an activity of 451 dpm as compared with 220 dpm in the cerebral white matter when calculated on a dpm/mg of dry weight basis. No comparable densitometer measurements of the films were made.

It is to be noted that those areas of localization of C-14 Isoniazid or its metabolites as indicated by the denser areas of the autoradiogram (Fig. 1) correspond to the density of the capillary bed in cortex and white matter of the cat brain, as measured by Hough and Wolff(5). To determine what relationship might exist between drug concentration and vascularity in the central nervous system, time studies with C-14 labeled Isoniazid and other radioactive compounds are in progress. I-131 labeled serum albumin, which is retained within the vascular system, will be used to determine the relative vascularity of various brain areas and the adequacy of the saline perfusion.

Summary. Gross autoradiograms of brain slices containing C-14 labeled Isoniazid, together with supportive data from radioactivity assay, are presented to show the differential localization of Isoniazid in brain tissue, and the technic is described. Future studies to determine the relationship between brain vascularity and brain drug content are indicated.

ADDENDUM: The authors have found that aluminized Mylar gives autograms that are superior to those prepared by using nonmetalized Mylar if more than 30 days exposure time is required.

Aluminized ¼ mil Mylar may be obtained from the Metalized Products Co., Inc., 29 Knight St., Norwalk, Conn.

1. Fitzgerald, Patrick, J., Simmel, Eva, Weinstein, Jerry, and Martin, Cynthia, *Lab. Invest.,* 1953, v2, 181.

2. Levi, Hilde, *Exp. Cell. Res.,* 1954, v7, 44.

3. Manthei, R. W., Roth, L. J., Barclay, W. R., and Ebert, R. H., *Arch int. pharmacodyn.,* 1954, v98, 183.

4. Murray, A., and Langham, W. H., *J. Am. Chem. Soc.,* 1952, v74, 6289.

5. Hough, H. B., and Wolff, H. G., *J. Comp. Neurol.,* 1939, v71, 427.

6. Hogness, J. R., Roth, L. J., Leifer, E., and Langham, W. H., *J. Am. Chem. Soc.,* 1948, v70, 3840.

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