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# ENTRY AND DISTRIBUTION OF HEXAMETHONIUM IN THE CENTRAL NERVOUS SYSTEM*

KHURSHEED ASGHAR and LLOYD J. ROTH

Department of Pharmacology, University of Chicago, Chicago, Ill. 60637, U.S.A.

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**Abstract**—Regional brain distribution of $^{3}H$- or $^{14}C$-labeled hexamethonium was studied after i.v. and intraventricular administration in rabbits and rats by radiochemical and autoradiographic techniques. Hexamethonium was found to penetrate into various brain regions within 5 min after i.v. administration in pharmacologically significant amounts with lower concentrations at 1 hr. Using cellular autoradiography, hexamethonium was seen in arachnoid-pia, and also associated with cellular elements of the CNS. After intraventricular administration, choroid accumulated a significantly higher concentration of hexamethonium than after i.v. administration. Choroid plexus appears to transfer hexamethonium by active transport from the CSF to the circulation.

THE QUATERNARY ammonium compounds, which in the body fluids exist exclusively in the charged form, are generally believed to enter the brain or the CSF poorly.[1–8] This argument has been used in the design of experiments in order to exclude the consideration of central effects of the quaternized compounds.[9] Because of the almost general acceptance of this concept, quaternary ammonium compounds are rarely screened for possible central pharmacological effects.

Levine[10] found hexamethonium and other quaternary ammonium compounds in the central nervous system (CNS) after i.v. administration to rats, while other authors[11,12] have found isotopically labeled decamethonium in the cat and chicken brain. [$^{14}C$]decamethonium was also seen by whole body autoradiography to penetrate the CNS within a few minutes after i.v. injection to mice.[13] In all the above studies, no correction was made for the contamination of the brain tissues by the blood contents, but Levine[10] indicated that the contribution of the blood to the amount found in brain tissue could not exceed 5 per cent of the total drug per gram of brain.

The studies of Roth *et al.*[14–18] indicated that when a correction was made for the blood contents either by brain perfusion with saline or by radio-iodinated serum albumin appreciable amounts of quaternary ammonium compounds were still found in the brain. After correction for the trapped blood contents, appreciable concentrations of [$^{14}C$]hexamethonium were found in the whole brain and spinal cord after an i.v. administration of 5 mg/kg to cats, and measurable concentrations were found in the CSF.[19] A previous study from this laboratory[20] reported that $^{14}C$-labeled hexamethonium enters the ventricular CSF of rabbits across the blood–CSF barrier after peripheral administration, under physiological conditions as well as under the conditions of cerebroventricular perfusion.

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Gosling and Lu[21] reported autoradiographic studies showing [$^3$H]hexamethonium and decamethonium only in choroid plexus and arachnoid. The radiochemical and autoradiographic studies reported herein indicate that measurable amounts of $^3$H-labeled hexamethonium enter the neuronal tissue in mice and rats, and substantiate the idea that the blood–brain extracellular fluid (ECF) interphase is not an absolute barrier to the penetration of hexamethonium.

## METHODS

*Materials.* [$^{14}$C] or [$^3$H]*N*-methyl-labeled hexamethonium and [$^{14}$C]carboxyl labeled dextran were obtained from New England Nuclear Corp. A radiopurity exceeding 99 per cent was reported by the supplier, which was confirmed in this laboratory by descending paper chromatography using the solvent system of *n*-butanol saturated with water and HCl (9:1). No radiochemical impurities were found. New Zealand albino male rabbits, weighing 0·9–1·6 kg (Abrams Laboratories), Holtzman white male rats, weight 140–200 g, and white mice, A/J strain weighing 20–25 g, were used.

*Penetration of [$^3$H]hexamethonium into rat brain.* A method to measure the contribution of the radioactivity from the blood to the total drug radioactivity in the brain has been described by Nair and Roth,[22] where one group of animals was injected with the drug and the other group with the radio-iodinated serum albumin (RISA-$^{131}$I). This method is based on the inability of RISA to penetrate the blood capillaries into the brain ECF[14,16,23] although the permeability to $^{125}$I-RISA has been reported to be altered in cerebral edema.[24] However, the method of Nair and Roth[22] suffers from the disadvantage of possible differences of cerebral blood contents at the time of sacrifice in the two groups of experimental animals.

$^{14}$C-labeled dextran with a molecular weight of about 50,000 is also not believed to penetrate the capillary–brain ECF barrier, and was administered i.v. concurrently with $^3$H-labeled hexamethonium to the same group of rats. The animals were sacrificed by freezing in liquid nitrogen 5 and 60 min after the administration of [$^{14}$C]dextran and [$^3$H]hexamethonium in a dose of 100 mg (40 or 100 $\mu$c) per kg and 10 mg (200 or 250 $\mu$c) per kg, respectively. Blood samples were obtained by heart puncture just prior to sacrifice. The brain tissue samples were dissected out from frozen brain, dried and weighed. This procedure was used by Nair *et al.*[14] and Nair and Roth,[15] and was followed in our studies to compare our results to theirs. The tissue samples up to 20 mg dry weight were solubilized either in Soluene (Packard Instrument Company) or in 2 N sodium hydroxide with subsequent addition of an equal volume of 4 N sodium acid phosphate. Dissolution of higher amounts of dried tissue samples leads to greater quenching of radioactivity counting. Soluene tissue solutions were incorporated in a phosphor solution of the following composition: 2,5-diphenyloxazole, 4 g; 1,4-bis[2-(5-phenyloxazolyl)] benzene, 400 mg; toluene, 1 l. All reagents were either analytical or scintillation grade. The samples were counted on a Packard Tricarb spectrometer with instrumental settings of 050-275, 40 per cent, and 050-1000, 12 per cent. The $^3$H and $^{14}$C dis./min values were calculated by the method of Okita *et al.*[25] The counting efficiencies under these conditions were determined by double internal standardization and were found to range between 3 and 10 per cent for $^3$H and between 10 and 50 per cent for $^{14}$C.

*Preparation of autoradiograms.* Whole body autoradiograms of rats and mice

were prepared by the technique of Ullberg,[26,27] as used by Asghar and Roth.[13] Methyl-$^{14}$C-labeled hexamethonium in saline solution was injected into the femoral vein of rats (10–20 mg/kg, approximately 2–10 $\mu$c) anesthesized with 50 mg kg of pentobarbital, whereas the mice receiving the drug i.v. or i.p. were previously unmedicated. Whole brain autoradiograms from rabbits were prepared at various intervals of time after i.v. administration of hexamethonium. For this study, the whole brain was dissected out within 5–7 min, the surface washed free of blood with chilled physiological saline (0°) and the brain frozen on dry ice. Coronal sections of brain ranging from 40 to 60 $\mu$ were cut and autoradiograms prepared in a manner similar to that described for whole body sections.

Cellular autoradiograms were prepared after the administration of *N*-methyl-$^{3}$H-labeled hexamethonium i.p. to mice in a dose of 30 mg/kg (2·5 $\mu$c/g) by the method of Stumpf and Roth.[28,29] No attempt was made to perfuse out the blood of the brain, since such a procedure might have produced diffusion of the polar hexamethonium molecules back into the vasculature perfusate. The dry mounting autoradiography procedure and its usefulness for the study of diffusible compounds have been discussed recently.[30–32] The freeze-dried sections of the cerebellum, cerebrum and diencephalon (2 $\mu$ thick) were exposed to dried photographic emulsion-coated slides for 8–10 weeks at −15°; the autoradiograms were developed and tissues were stained with hematoxylin and eosin, periodic acid-Schiff reagent and methylgreen pyronin.

## RESULTS

*Correction for brain blood radioactivity.* The validity of the rationale of using [$^{14}$C]dextran to make a correction for the contribution of the circulation to the total drug contents of the brain is shown in Table 1, where the "relative penetration" of [$^{14}$C]dextran is compared to that of $^{131}$I-RISA. The relative penetration of [$^{14}$C]dextran was measured at 5 min and 1 hr in the various brain regions studied, including cerebral cortex, basal ganglia, thalamus, hippocampus, inferior colliculi, cerebellar cortex and medulla (Table 1). The differences between the [$^{14}$C]dextran and [$^{131}$I]RISA space were not significant.

The comparison of the relative penetration of [$^{3}$H]hexamethonium with [$^{131}$I]RISA and sulfate (Table 1) indicates that in some regions, e.g. basal ganglia, inferior colliculi and pons-medulla, hexamethonium space is even higher than sulfate space, suggesting that hexamethonium may not be restricted to the extracellular space of the brain and might have actually penetrated the neuronal tissue.

If there were no penetration of [$^{3}$H]hexamethonium into the brain, the ratio of $^{3}$H dis./min to $^{14}$C dis./min found in brain tissue, including its vascular contents, should be equal to that of the radioactivity in the blood at the time of sacrifice. The total $^{3}$H and $^{14}$C activity found in the brain samples of a rat, after concurrent administration of [$^{3}$H]hexamethonium and [$^{14}$C]dextran, is shown in Table 2. The ratio of $^{3}$H dis./min to $^{14}$C dis./min in whole blood or plasma at the time of sacrifice (1 hr) was 3·06. Column C of Table 2 shows the dis./min (3·06 × $^{14}$C dis./min) which should be expected, assuming no penetration of hexamethonium in the brain. Thus, any radioactivity exceeding this value (column D of Table 2) can be considered to have diffused across the blood–brain ECF barrier. From this, the absolute amount of drug (column E of Table 2) can be calculated.

TABLE 1. COMPARISON OF RELATIVE PENETRATION* OF [$^{131}$I]RISA AND [$^{14}$C]DEXTRAN AND [$^{3}$H]HEXAMETHONIUM IN VARIOUS BRAIN REGIONS AT 5 min AFTER I.V. ADMINISTRATION

| Samples from the region of | [$^{131}$I]RISA† (15) | [$^{35}$S]sulfate† (6) | [$^{14}$C]dextran (3) | [$^{3}$H]hexamethonium (3) |
|---|---|---|---|---|
| Basal ganglia | 5·53 ± 0·11‡ | 5·8 ± 0·66‡ | 4·1 ± 0·5 | 49·1 ± 5·5 |
| Cerebral cortex | 8·49 ± 0·59 | 21·0 ± 2·24 | 9·1 ± 0·9 | 45·3 ± 10·5 |
| Hippocampus | 5·38 ± 0·36 | 7·5 ± 0·09 | 5·7 ± 1·0 | 27·9 ± 5·5 |
| Thalamus | 5·67 ± 0·13 | 5·6 ± 0·43 | 5·3 ± 0·4 | 24·4 ± 6·7 |
| Inferior colliculi | 8·32 ± 0·63 | 12·2 ± 1·45 | 8·4 ± 0·5 | 23·0 ± 2·7 |
| Cerebellar cortex | 10·95 ± 0·68 | 13·8 ± 0·96 | 9·9 ± 2·0 | 21·3 ± 7·1 |
| Pons-medulla | 5·25 ± 0·35§ | 7·9 ± 0·30§ | 4·4 ± 0·5 | 25·8 ± 13·2 |

* The figures are means of $\frac{\mu g \text{ of drug/g dry weight}}{\mu g \text{ of drug/ml blood}} \times 100$; when a solute is restricted to the vascular space, this ratio represents $\mu$l of blood per 100 mg of tissue (dry weight). The numbers in parentheses represent the animals used to obtain that value.

† RISA and sulfate values are taken from Nair *et al.*[14] and Nair and Roth[15] respectively.

‡ Caudate nucleus.

§ Medulla.

TABLE 2. ILLUSTRATION OF THE APPLICATION OF THE CORRECTION FOR BLOOD RADIOACTIVITY TO THE TOTAL DRUG CONTENTS IN BRAIN SAMPLES OF AN INDIVIDUAL RAT*

| Sample from the region of | A Total $^{3}$H (dis./min/g × $10^2$) | B Total $^{14}$C (dis./min/g × $10^2$) | C† $^{3}$H, assuming no penetration (dis./min/g × $10^2$) | D Amount penetrated (A−C) (dis./min/g × $10^2$) | E‡ ($\mu$g/g) |
|---|---|---|---|---|---|
| Olfactory lobes | 3504 | 364 | 1114 | 2390 | 0·43 |
| Neocortex | 1693 | 298 | 912 | 781 | 0·14 |
| Corpus callosum | 1223 | 19·2 | 59 | 1164 | 0·21 |
| Cerebral cortex | 883 | 36·1 | 110 | 773 | 0·14 |
| Hippocampus | 667 | 29·2 | 89 | 578 | 0·146 |
| Geniculate bodies | 1448 | 17·3 | 53 | 1435 | 0·26 |
| Inferior colliculi | 1754 | 119 | 364 | 1390 | 0·25 |
| Cerebellar cortex | 2035 | 169 | 517 | 1518 | 0·27 |

* Dose: [$^{3}$H]hexamethonium dichloride, 10 mg/kg, 200 $\mu$c/kg; [$^{14}$C]dextran, 100 mg/kg, 40 $\mu$c/kg. The concentrations of hexamethonium and dextran are expressed per gram of dry tissue weight.

† Plasma $^{3}$H/$^{14}$C at 1 hr = 3·06; column C = 3·06 × column B.

‡ Specific radioactivity = 5·55 × $10^5$ dis./min/$\mu$g of hexamethonium dichloride.

*Determination of the amount of [$^{3}$H]hexamethonium in various brain regions.* The amount of [$^{3}$H]hexamethonium penetrating the various brain regions at 5 min and 1 hr after a concurrent i.v. administration of 10 mg/kg of [$^{3}$H]hexamethonium and 100 mg/kg of [$^{14}$C]dextran is shown in Table 3. The concentrations of hexamethonium in various brain regions shown in the above table have been corrected for the contribution of the radioactivity from the blood present in the vasculature of the brain at the time of sacrifice. Invariably, in all the brain regions examined, the concentration of [$^{3}$H]hexamethonium in the brain at 5 min is higher than that present at 1 hr. The mean levels of hexamethonium (dichloride) were 2·86 and 1·0 $\mu$g/ml in blood obtained from

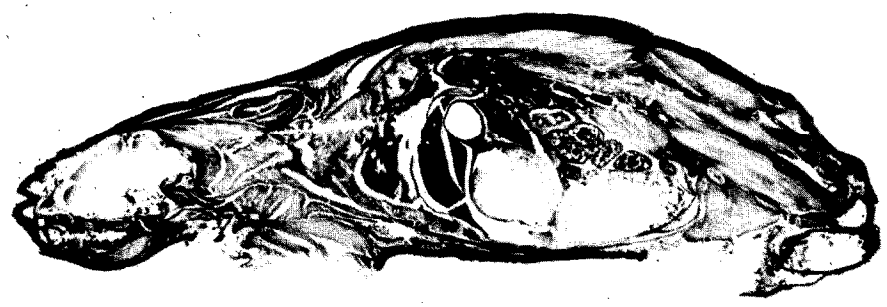

FIG. 1. Rat whole body autoradiogram showing the distribution of [$^{14}$C]hexamethonium in the CNS and other organs of the body 60 min after i.v. administration of 10 mg/kg of hexamethonium dichloride. The dark areas indicate radioactivity.

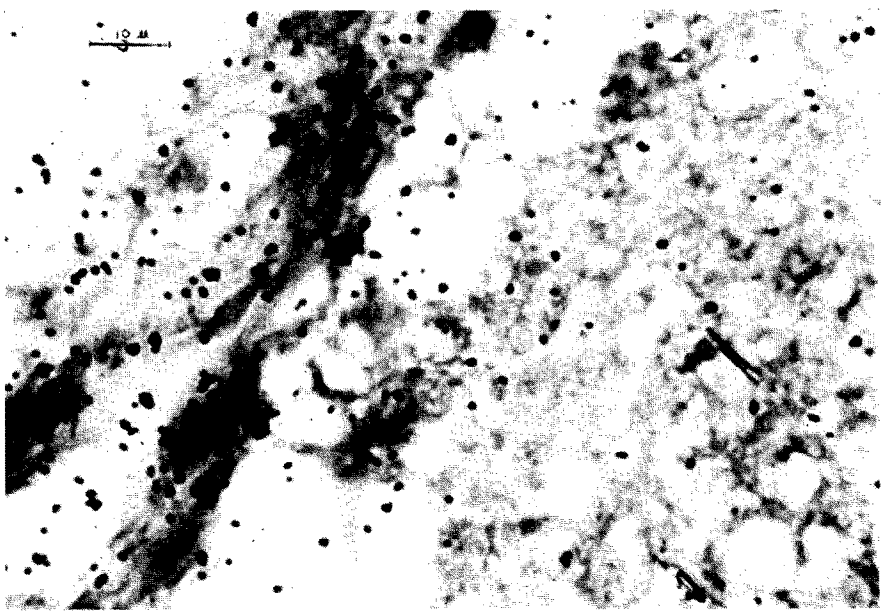


FIG. 2. Cellular autoradiogram showing the molecular layer of cerebellar cortex and part of the choroid plexus in the fourth ventricle of a mouse 100 min after i.p. administration of 30 mg/kg of [$^{3}$H]labeled hexamethonium dichloride. Note the relative activities in choroid and the cerebellar cortex. Stain: methylgreen pyronine (×325). Methylgreen pyronine specifically stains DNA and RNA. Tissue areas containing mainly lipid and carbohydrate constituents remain unstained.

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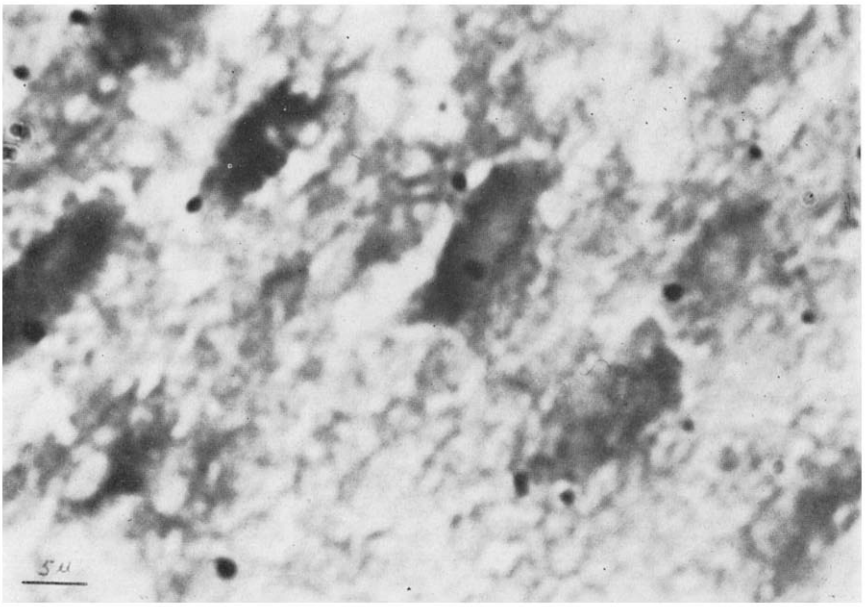


FIG. 3. Cellular autoradiogram showing the distribution of silver grains under the stellate cells 100 min after i.p. administration of 30 mg/kg of [$^3$H]labeled hexamethonium dichloride. Stain: methylgreen pyronin. (×504).

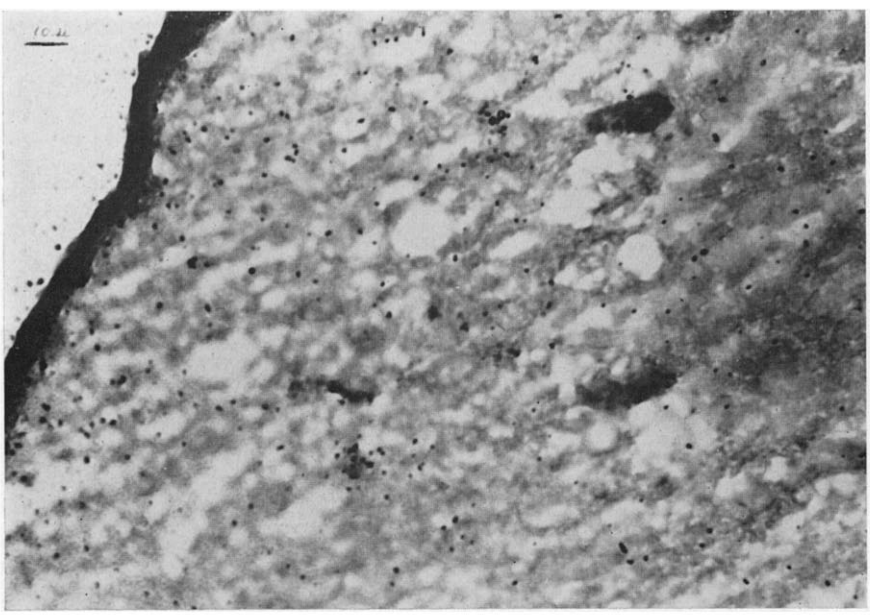

FIG. 4. Cellular autoradiogram showing the distribution of [$^3$H]hexamethonium in the arachnoid-pia and in the molecular, Purkinje cells and granular layers of the cerebellar cortex 100 min after i.p. administration of 30 mg/kg of hexamethonium dichloride to a mouse. Stain: methylgreen pyronin (×160).

TABLE 3. REGIONAL BRAIN DISTRIBUTION OF [$^3$H]HEXAMETHONIUM IN RAT CORRECTED FOR THE CONTRIBUTION OF RADIOACTIVITY FROM THE BLOOD PRESENT IN THE BRAIN

| Tissue | [$^{14}$C]hexamethonium dichloride ($\mu$g/g dry wt.)* | |
|---|---|---|
| | 5 min | 60 min |
| Basal ganglia | 1·41 ± 0·07 | 0·68 ± 0·37 |
| Choroid | 0·77 ± 0·66 | 0·03 ± 0·02 |
| Cerebral cortex | 1·30 ± 0·27 | 0·13 ± 0·09 |
| Hippocampus | 0·80 ± 0·14 | 0·08 ± 0·03 |
| Thalamus | 0·70 ± 0·18 | 0·09 ± 0·04 |
| Inferior colliculi | 0·66 ± 0·04 | 0·42 ± 0·22 |
| Cerebellar cortex | 0·61 ± 0·18 | 0·20 ± 0·05 |
| Cerebellum | 0·39 ± 0·15 | 0·36 ± 0·24 |
| Pons-medulla | 0·74 ± 0·37 | 0·22 ± 0·11 |

* All the values shown above are the means of three animals, ± the standard error of the mean (S.E.M.).

the heart at 5 and 60 min respectively. The highest drug concentration at 5 min was found in the basal ganglia with decreasing amounts in the cerebral cortex, hippocampus, pons-medulla, thalamus, inferior colliculi and cerebellar cortex. Additional regions which were not analyzed in all the animals, such as olfactory lobes, also contained relatively high concentrations of [$^3$H]hexamethonium. At 5 min, the choroid plexus was found to contain appreciable amounts of [$^3$H]hexamethonium when corrected for its vascular content. However, at 1 hr, almost all the activity found in the choroid plexus was due to the presence of blood in the vasculature. The paraventricular area in the cerebrum also contained significantly lower amounts of the labeled drug at 1 hr as compared to that at 5 min. Wide differences in the brain concentration of hexamethonium were observed from one animal to another; these differences are reflected in the standard errors.

After intracerebroventricular perfusion of $10^{-5}$ M (2·73 $\mu$g/ml) hexamethonium in synthetic CSF for about 90 min, the choroid plexus contained the highest amount of hexamethonium (dichloride), i.e. 13·22 $\mu$g/g, among various areas of rabbit brain, and was markedly higher than the choroid concentration attained after i.v. administration of 15 mg kg of [$^{14}$C]hexamethonium to a rabbit. In rats the concentrations observed in the cellular elements of the choroid were also relatively lower as compared to those of some other brain regions after i.v. injection of hexamethonium (Table 3); the concentrations were further decreased at 1 hr as compared to 5 min.

*Autoradiographic studies.* The whole body autoradiograms were prepared from rats and mice at intervals ranging from 15 min to 30 hr after the i.v. or i.p. administration of 10 mg/kg of [$^{14}$C]hexamethonium. A typical distribution pattern of labeled hexamethonium as obtained in a whole body autoradiogram is shown in Fig. 1. The highest radiodensity was found localized in kidney, urinary bladder and intestine, skin and cartilage. There is some radiodensity in the cerebral and cerebellar cortex and in the choroid plexus of the fourth ventricle. Autoradiograms prepared from coronal sections of rabbit brain after i.v. administration of 15 mg kg of labeled drug also show the radiodensity present in highly vascular areas such as cerebral and

cerebellar cortex, choroid plexus, and areas which are adjacent to the ventricular space.

After intracerebroventricular administration to rats of 20 $\mu$l of solution containing 100 $\mu$g of labeled hexamethonium, radioactivity spread throughout the brain and the vertebral column. Hexamethonium, when given intraventricularly in this high concentration, was seen in autoradiograms to penetrate most of the brain areas. Relatively little drug was seen in the rest of the rat body, except in the kidney and the urinary bladder, indicating transference of the labeled drug from the cerebroventricular space into the circulation from which it is preferentially accumulated by the kidney.

Cellular autoradiograms prepared from 2·0 $\mu$ thick sections of the cerebellum and other parts of the brain can be used for the cellular localization of the labeled drug in the brain [28, 29, 32]. Cerebellar cortex and the choroid plexus around the fourth ventricle of a mouse are shown in Fig. 2. The abundance of silver grains in the choroid plexus capillaries is associated with the presence of blood. While most of the activity appears in the choroidal capillaries, an appreciable fraction is also seen under the cells of the choroid. This conclusion is justified on the basis of the resolution studies of Brown *et al.*,[33] indicating that 90 per cent of the silver grain density in the photographic emulsion from [$^3$H]thymidine incorporated into nuclear DNA is found within a distance of approximately 0·5 $\mu$ from the nuclear border. In contrast, relatively little activity was found in the molecular layer of the cerebellar cortex. It may be noted that an appreciable proportion of radioactivity can be seen in the cells, including stellate, Purkinje and granule cells of the cerebellar cortex (Fig. 3). Cerebellar white matter, however, was found to have little radioactivity. Besides the choroid plexus, the meninges, e.g. arachnoid and pia of the brain, seem to be particularly rich in the labeled hexamethonium (Fig. 4). Further, the external layers of the brain, e.g. the outer cerebellar molecular layer, had a greater number of silver grains than the cerebellar granular layer or the white matter.

## DISCUSSION

Hexamethonium is a water-soluble polar diquaternary ammonium drug which, in the body fluids, exists exclusively in the dicationic form. Hexamethonium is not metabolized in mice over a period of at least 18 hr[34] and, in rabbits, no degradation products have been found in the urine.[35] The radioactivity measured in the radiochemical analyses and the radiodensity observed on the autoradiograms, therefore, may be interpreted to reflect the relative concentration of the original compound injected.

Radiochemical studies using [$^{14}$C]dextran and [$^3$H]hexamethonium indicate that [$^3$H]hexamethonium diffuses across the capillary–brain ECF barrier and enters the neuronal and cellular elements of the brain. The choroid plexus concentration of hexamethonium attained after intraventricular administration was found to be higher than that after i.v. administration of hexamethonium. After i.v. administration, most of the hexamethonium in the choroid was found to be in the vasculature, while after ventricular perfusion, the concentration of [$^3$H]hexamethonium in the choroid was considered to be in the cellular elements and ECF. Since the highest concentrations of hexamethonium in the choroid were found only after intraventricular administration, the active transport of hexamethonium by choroid[36] appears to take place only in the

direction of CSF to blood, and the low levels of hexamethonium found in the CNS after i.v. administration may be a reflection of its rapid active removal from the ventricular CSF.[20]

In cellular autoradiographic studies, the brain vasculature was not perfused with saline to remove the blood radioactivity before sectioning and subsequent preparation of autoradiograms, since such a perfusion may cause diffusion or leakage of polar hexamethonium from the brain tissue. The cellular autoradiographic studies show that the radioactivity, sensed by the emulsion, lies in the neuronal tissue including stellate, Purkinje, granule and pyramidal cells of the brain. Arachnoid contains the highest activity in the CNS. The presence of activity inside the choroidal cells depends upon the route and time lapse after administration. The radiochemical data indicate penetration of the labeled hexamethonium into CNS and CSF immediately after administration.

The concentration gradient of the silver grains in the autoradiographic emulsion under cerebral and cerebellar cortex suggests that one of the routes of penetration of hexamethonium into brain cells may be across the blood capillary–CSF barrier, located partly in the choroid plexus, with consequent diffusion into the paraventricular neuronal tissue. This idea is substantiated by the immediate penetration of the labeled hexamethonium into the CSF after i.v. administration.*

Gosling and Lu[21] could not detect any hexamethonium in regions of CNS other than the meninges and the choroid 4 hr after an i.m. dosage of 0·8 ml/kg of $10^{-2}$ M hexamethonium solution, which is equivalent to 2·18 mg/kg of hexamethonium dichloride. Since the ganglionic blocking dose of hexamethonium dichloride in rats is about 20 mg/kg i.v.,[37] we administered 10–30 mg/kg of the drug by i.p. and i.v. injection. The observed differences, probably, are the result of the hexamethonium dosage, time elapsed after drug administration, and the autoradiographic technique used by Gosling and Lu,[21] in which the autoradiograms were prepared by exposing undried frozen tissue sections to the photographic emulsion, where possible translocation of hexamethonium and negative artifact could take place.[30,32]

Whole body or whole brain autoradiography does not reveal whether the drug is present in the blood vessels or the brain cells, nor can it unequivocally show the nonpenetration of any drug into brain or any other region. The whole body or whole organ autoradiograms only shows the relative distribution of a drug, and prolonged exposure of the whole body sections to the X-ray films produces darkening of film areas corresponding to the tissue areas containing relatively less activity. On the other hand, relatively shorter duration of exposure might show high radiodensity in some areas, with brain appearing completely devoid of activity. Therefore, the argument of nonpenetration of quaternary ammonium compounds based on autoradiograms used in some studies[7,8] is not justified.

The classical concept of nonpenetration of quaternary ammonium compounds in the CNS is partly based on the relatively less sensitive techniques of the past, and partly because of the acceptance of "poor" penetration of these drugs: choline,[38] bretylium,[39] 2-PAM,[18,40] promethazine methiodide and mepazine methiodide,[41] and hemicholinium-3,[42] where the poor penetration did not appear to be due to the impermeability of the blood–CNS barrier, but rather was due to the strong binding of hemicholinium to the red blood cells.

* Asghar and L. J. Roth, unpublished data.

The fact that various quaternary ammonium compounds produce a marked pharmacological response, sometimes similar to that of their tertiary analog, after direct introduction into discrete loci in the CNS is well known: atropine methylbromide,[43] quaternized thiazine and thioxanthenes including quaternized chlorpromazine,[44] *N*-methyl morphine,[45] hemicholinium-3[46,47] and hexamethonium.[48] Therefore, even a poor penetration of a compound into the CNS which is centrally active could be pharmacologically significant, since several quaternary ammonium compounds have been reported to have presumably a central pharmacological action after peripheral administration: 2-PAM[40,49–51] and hemicholinium-3.[52–57] Further, the central actions of the quaternary derivatives of nicotine also suggest the possibility of their penetration into the CNS.[58]

## REFERENCES


1. W. D. M. PATON and E. J. ZAIMIS, *Pharmac. Rev.* **4,** 219 (1952).
2. B. B. BRODIE and A. M. HOGBEN, *J. Pharmac. exp. Ther.* **9,** 345 (1957).
3. A. S. V. BURGEN and L. M. CHIPMAN, *Q. Jl exp. Physiol.* **37,** 61 (1951).
4. B. B. BRODIE, H. KURZ and L. S. SCHANKER, *J. Pharmac. exp. Ther.* **130,** 20 (1960).
5. L. S. SCHANKER, *Pharmac. Rev.* **14,** 501 (1962).
6. E. N. COHEN, *J. Pharmac. exp. Ther.* **141,** 356 (1963).
7. E. HANSSON and C. G. SCHMITERLOW, *Acta Pharmac. tox.* **18,** 183 (1961).
8. E. HANSSON and C. G. SCHMITERLOW, *Archs int. Pharmacodyn. Thér.* **131,** 309 (1961).
9. E. F. DOMINO and M. E. OLDS, *J. Pharmac. exp. Ther.* **164,** 202 (1968).
10. R. LEVINE, *Nature, Lond.* **184,** 1412 (1959).
11. U. LUTHI and P. G. WASER, *Archs int. Pharmacodyn. Ther.* **156,** 319 (1965).
12. T. C. LU, J. A. GOSLING and D. B. TAYLOR, *Eur. J. Pharmac.* **3,** 364 (1968).
13. K. ASGHAR and L. J. ROTH, *J. Pharmac. exp. Ther.* **176,** 83 (1971).
14. V. NAIR, D. PALM and L. J. ROTH, *Nature, Lond.* **188,** 497 (1960).
15. V. NAIR and L. J. ROTH, *Radiat. Res.* **23,** 249 (1964).
16. V. NAIR, H. LAL and L. J. ROTH, *Int. J. Neuropharmac.* **1,** 361 (1962).
17. L. J. ROTH and C. F. BARLOW, *Isotopes in Experimental Pharmacology*, p. 49. University of Chicago Press, Chicago (1964).
18. H. FIREMARK, C. F. BARLOW and L. J. ROTH, *J. Pharmac. exp. Ther.* **145,** 252 (1964).
19. R. J. MCISAAC, *J. Pharmac. exp. Ther.* **135,** 335 (1962).
20. K. ASGHAR and L. J. ROTH, *Fedn Proc.* **28,** 578 (1969).
21. J. A. GOSLING and T. C. LU, *J. Pharmac. exp. Ther.* **167,** 56 (1969).
22. V. NAIR and L. J. ROTH, *Isotopes in Experimental Pharmacology*, p. 219. University of Chicago Press, Chicago (1964).
23. N. B. EVERRETT and B. SIMMONS, *Circulat. Res.* **6,** 307 (1958).
24. R. W. P. CUTLER, G. V. WATTERS and C. F. BARLOW, *Archs Neurol.* **11,** 225 (1964).
25. G. T. OKITA, J. J. KABARA, F. RICHARDSON and G. V. LEROY, *Nucleonics* **15,** 111 (1957).
26. S. ULLBERG, *Acta Radiol.* **supp. 118** (1954).
27. S. ULLBERG, *Proc. Second Int. Conf. on the Peaceful Uses of Atomic Energy, Geneva*, Vol. 24, p. 248. I.D.S., Columbia University Press, New York (1958).
28. W. E. STUMPF and L. J. ROTH, *J. Histochem. Cytochem.* **15,** 243 (1967).
29. W. E. STUMPF and L. J. ROTH, *Advances in Tracer Methodology*, Vol. 4, p. 113. Plenum Press, New York (1968).
30. L. J. ROTH and W. E. STUMPF, Eds., *Autoradiography of Diffusible Substances*, p. 1. Academic Press, New York (1969).
31. W. E. STUMPF, *J. Histochem. Cytochem.* **18,** 21 (1970).
32. L. J. ROTH, *Handbuch der Exp. Pharmakologie, Bd.* 28, p. 286. Springer-Verlag, Heidelberg (1971).
33. D. A. BROWN, W. E. STUMPF, I. M. DIAB and L. J. ROTH, *Autoradiography of Diffusible Substances*, p. 161. Academic Press, New York (1969).
34. R. R. LEVINE and B. B. CLARK, *Fedn Proc.* **16,** 317 (1957).
35. E. J. ZAIMIS, *Br. J. Pharmac.* **5,** 424 (1950).
36. Y. TOCHINO and L. S. SCHANKER, *Am. J. Physiol.* **208,** 666 (1965).
37. G. C. SALMOIRAGHI, J. W. MCCUBBIN and I. H. PAGE, *J. Pharmac. exp. Thér.* **119,** 240 (1957).
38. J. E. GARDINER and F. R. DOMER, *Archs int. Pharmacodyn. Thér.* **76,** 482 (1968).

39. A. L. A. Boura, F. C. Copp, W. G. Duncombe, A. F. Green and A. McCoubrey, *Br. J. Pharmac.* **15,** 265 (1960).
40. P. Aarseth and J. A. B. Barstad, *Archs int. Pharmacodyn. Thér.* **76,** 434 (1968).
41. C. L. Huang, J. A. Yeh and S. Y. Hsu, *J. pharm. Sci.* **59,** 772 (1970).
42. F. R. Domer and F. N. Schueler, *J. pharm. Sci.* **49,** 553 (1960).
43. K. A. Khavari and R. P. Maickel, *Int. J. Neuropharmac.* **6,** 301 (1967).
44. R. L. Fog, A. Randrup and H. Pakkenberg, *Psychopharmacologia* **12,** 428 (1968).
45. R. S. Foster, P. Lomax and D. J. Jenden, *Proc. west. Pharmac. Soc.* **10,** 34 (1967).
46. A. T. Dren and E. F. Domino, *J. Pharmac. exp. Ther.* **161,** 141 (1968).
47. C. O. Hebb, G. M. Ling, E. G. McGeer, P. L. McGeer and D. Perkins, *Nature, Lond.* **204,** 1309 (1964).
48. N. Fadhel and L. D. Seager, *Proc. Soc. exp. Biol. Med.* **131,** 1263 (1969).
49. V. G. Longo, D. Nachmansohn and D. Bovet, *Archs. int. Pharmacodyn. Thér.* **123,** 282 (1960).
50. R. V. Brown, A. M. Kunkel, L. M. Somers and J. H. Wills, *J. Pharmac. exp. Ther.* **120,** 276 (1957).
51. P. Rosenberg, *Biochem. Pharmac.* **3,** 212 (1960).
52. Y. Kase and H. L. Borison, *J. Pharmac. exp. Ther.* **122,** 215 (1958).
53. H. L. Borison, *Fedn Proc.* **20,** 594 (1961).
54. L. E. McCarthy, G. Johnson, R. Taylor and H. L. Borison, *Pharmacologist* **4,** 182 (1962).
55. B. Metz, *Am. J. Physiol.* **202,** 80 (1962).
56. D. T. Frazier and L. L. Boyarsky, *Proc. Soc. exp. Biol. Med.* **115,** 876 (1964).
57. J. C. Szerb, *Nature, Lond.* **197,** 1017 (1963).
58. R. B. Barlow, A. Oliverio, M. Satta and G. M. Thompson, *Br. J. Pharmac.* **39,** 647 (1970).