THE SITE OF ACTION OF PHENOTHIAZINE S^{35} IN THE CENTRAL NERVOUS SYSTEM

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INTRODUCTION

THE role of phenothiazine derivatives in controlling the symptoms of mental diseases has been investigated by many workers by determining its site of action in the central nervous system.

Physiological experiments in man and animals conducted by Bremer and Terzoulo (1953), Cazzullo and Guareschi (1954) and Courvoisier *et al.* (1953) suggested a cortical action.

The action of the drug on the diencephalon was suggested by Dasgupta and Werner (1954) and Cathala and Pocidalo (1953) by experimenting on decorticated cats and monkeys.

EEG Studies in man and animals were carried out by Terzian (1952), Carreras and De Risio (1952), Carreras and Angeleri (1957), Dasgupta and Werner (1954), Hiebel *et al.* (1954), Balestrieri and Fadiga (1954) and De Risio and Manghi (1954), using chlorpromazine. They indicated that the main site of action of the drug is on the reticular formation.

Pharmacological studies of different phenothiazine compounds on animals were reported by Zakrzewska (1957), Arrigoni-Martelli *et al.* (1958), Bray and Funkenstein (1958), Brunson *et al.* (1959) and Tislow (1961). These studies pointed to the nuclei of the brain stem either in the reticular formation or vasomotor vegetative centres as sites of action. A combined electroencephalographic and pharmacological study done by Martin and Eades (1960) has indicated that chlorpromazine and sulphoxide-compounds decrease the excitability of both the ascending activating system and descending vasomotor system.

Few chemical studies on the distribution of the drug in the various brain areas have been reported by Wase *et al.* (1956), Federov (1958), Salzman and Brodie (1956), Berger, Wechsler and Roisin (1960). The first two used phenothiazine derivatives with marked atoms ; the majority were unable to demonstrate any significant difference in the concentration of the drug in different brain areas. Wase *et al.*, however, reported slight higher levels in chlorpromazine S^{35} in the first day after injection in mice in hypothalamus, thalamus and brain stem than in cerebellum and still less in cortex. Wechsler and Roisin found higher concentration in cortex and basal ganglia than in spinal cord, cerebellum and pons.

In the present state of uncertainty as to the elective site of action of chlorpromazine, this work is an attempt at the determination of the distribution of phenothiazine in the brain utilizing the radioactive labelling technique.

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Materials

Experimental animals: 4 cats
1 monkey
1 human being (advanced carcinoma of bladder with terminal uraemia)

Radioactive drug: Phenothiazine S^{35}

Methods

Dose given for cat: 50 mc. S^{35}
Dose given for monkey: 100 mc. S^{35}
Dose given for human being: 200 mc. S^{35}

The above-mentioned doses were given intravenously to each of the experimental animals or patient.

Cats were sacrificed after 20 minutes 2 hours and 24 hours, while the monkey was sacrificed after 2 hours. The human being was given a dose 28 hours before death after the consent of his relatives.

The brain was dissected immediately after death, washed in saline several times and separated into medulla, pons, midbrain, thalamus and hypothalamus, frontal, temporal and parieto-occipital lobes and cerebellum.

These parts were separately digested in concentrated nitric acid so as to dissolve it completely. The acid solution was made up to standard volume (100 ml.). 1 ml. liquid was taken for evaporation in aluminium cups by infra-red lamp. Samples were weighed, counted, corrected for self absorption and counting errors. The specific activity was calculated for 1 gm. of the solid residue and the results were tabulated.

Auto-radiography

The apposition technique was followed for the auto-radiography of the brain stem. The brain stem was dissected 2 hours after the injection of the dose, fixed in 10% formaldehyde and bisected into two equal longitudinal halves. Each half was mounted in paraffin and serial sections were taken by the microtome amounting to 200 sections for each half. The sections were placed on dental films for a week and then developed, fixed and compared.

RESULTS

TABLE I

The Percentage Uptake of Phenothiazine S^{35} in Different Parts of the Brain

Animal	Time after injection	Specific Activity (cps/gm. dry wt.)							
		Medulla	Pons	Mid-brain	Thalamus and Hypo-thalamus	Frontal lobe	Pariet-occip. lobe	Temporal lobe	Cere-bellum
Cat ...	20 min.	154	76·7	47·7	33·5	138	153	97·9	25
Cat ...	2 hr.	558	236	420	344	188	267	176	136
Cat ...	24 hr.	77·3	84·1	72·6	18·4	21·5	21·3	158·3	42
Monkey ...	2 hr.	71·1	36	126	11·1	12·6	4	19	11
Human patient	28 hr.	30	27	8	28	20	50	23·6	40

It is shown from Table I that a rapid uptake in the medulla and cortex is noted 20 minutes after injection. Blood sample was taken directly before sacrificing the first animal (cat, 20 min. after injection), for plasma counts. The specific activity was found to be 600·6 cps/ml. plasma. Such a result indicated that the uptake in the different parts of the brain was specific and did not represent passive diffusion or simply radioactivity in the brain by virtue of its blood supply.

In cat and monkey the distribution of the radioactive drug is more localized in the different parts of the brain stem 2 hours after injection compared

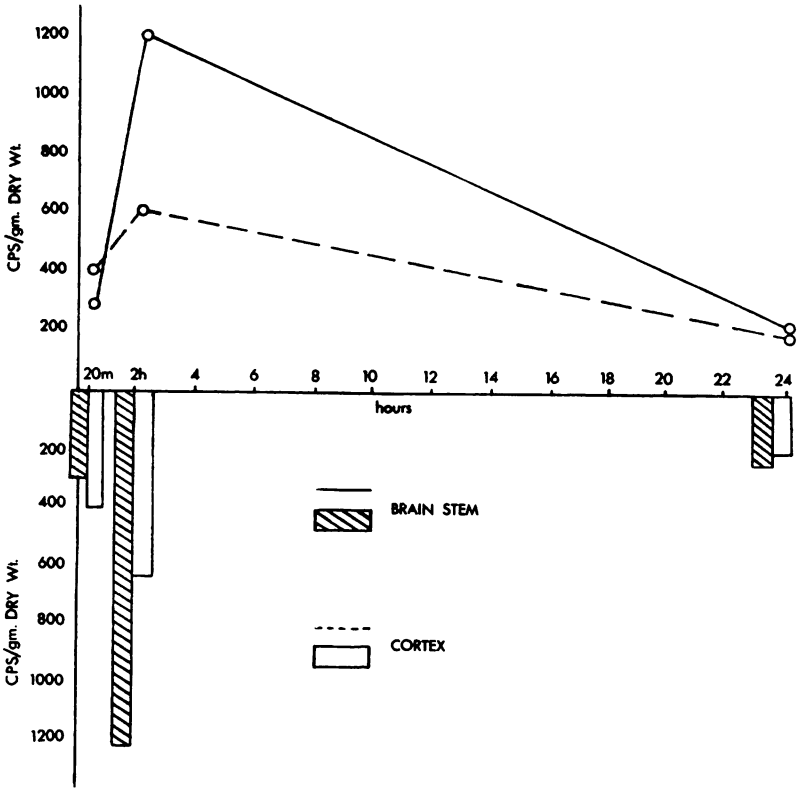

FIG. 1
Curve and bar showing uptake of drug in cat at different times comparing brain stem and cortex.

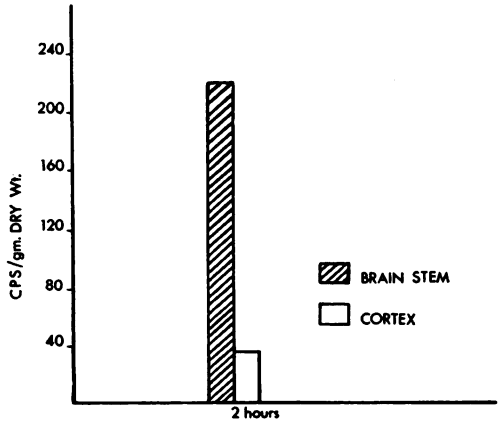

FIG. 2
Bar showing uptake of drug in monkey two hours after injection comparing brain stem and cortex.

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to the other parts of the brain. Another observation shows that the drug is more rapidly eliminated from the brain stem than from the cortex (see Table II), figs. 1 and 2. The drug was least concentrated in the thalamus and hypothalamus as well as the cerebellum.

Seing that the uptake of the drug was more pronounced in the brain stem as shown in Table II, an auto-radiographic experiment was carried out to show the exact site of concentration of the drug in that part. The results obtained by dental films show that most of the drug was localized in the central quarter of the brain stem, these were the medial 50 serial sections on either longitudinal halves. As we go from the medial part to the lateral part, the uptake gradually diminished (fig. 3).

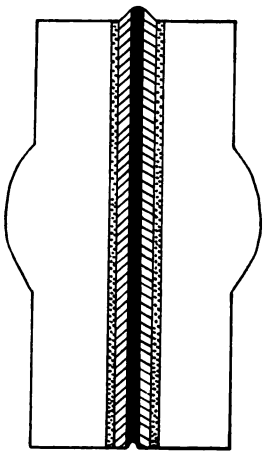

FIG. 3
Diagram of brain stem demonstrating concentration of the drug by autoradiography in the central quarter only. Different shades show degrees of concentration.

TABLE II

Animal	Time after injection	Specific Activity (cps/grm. dry wt.)	
		Brain Stem	Cortex
Cat	20 mins.	278·4	388·9
Cat	2 hours	1214·0	621·4
Cat	24 hours	234·0	201·6
Monkey	2 hours	229·7	35·1
Human patient	28 hours	65	93·6

DISCUSSION

From the results obtained in cat and monkey we came to the following conclusions. Firstly, the drug is mostly concentrated in brain stem, and to a lesser extent in cortex and least in thalamus, hypothalamus and cerebellum. Secondly, in cat during the first 20 minutes after injection, the drug is more concentrated in the cortex compared to the brain stem. This can be correlated with the relative vascularity. Barlow *et al.* (1957) obtained data on the relative vascularity in the various structures of the cat brain by densitometric measurement of auto-radiograms after injection of I^{131} labelled serum albumen. They shewed that the cerebral cortex and thalamus including the geniculate bodies are more

vascular than the brain stem. This, however, cannot represent the whole truth concerning the disproportionate distribution of the drug through various brain areas because, although the cerebellum and thalamus rank on top of the list with the cerebrum, yet they received the least amount of the drug. This might imply an affinity of the cortex to the drug more than can be accounted for by the distribution of the vascularity. The uptake of the drug in the medulla which is less vascular is highest compared to other single structures of the brain. Thirdly, as shown in Table II the rate of uptake in the brain stem to the cortex of the cat is 2 : 1 at the 2-hour period. This indicates marked affinity of the brain stem to the drug at that time. Comparing the 2–24 hours period it is noted that the rate of elimination of the drug in the brain stem is 6 : 1 while in the cortex it is 3 : 1. These observations indicate that the drug is not only taken up mainly by the brain stem but is more utilized by the same part. Fourthly, the results of auto-radiography prove that the drug is selectively taken in the middle quarter of the brain stem which corresponds to the reticular formation. Further auto-radiography for the antero-posterior dimension would have been attempted had available drug been at hand.

The behaviour of the drug in cat and monkey is similar. In both the ratio of uptake of the drug in brain stem to cortex shows a more selective uptake in the former, particularly in the monkey (2 : 1 in the cat and 6 : 1 in the monkey). Such comparative result in experimental animals are feasible because of the capability of sacrificing the animal at the chosen time. Considering that the monkey represents an ascending grade in evolution of the animal kingdom, one might feel justified in predicting the probable result of uptake of the drug in the various parts of brain of man by inference from results obtained in the monkey. If we apply the scale of elimination of the drug in the brain stem and cortex of the cat in Table II between the 2 hours and 24 hours period, to the figures obtained in man after 24 hours of injection, one can deduce that the concentration of the drug in the brain stem in man would have been higher at the 2 hours period. (Brain stem would be : $65 \times 6 =$ about 400 ; Cortex would be : $93.6 \times 3 =$ about 280).

Our comparative data were taken 2 hours after injection because the optimal clinical effect of the drug in the human being is obtained by that time. The failure to demonstrate significant difference in the concentration of the drug on various brain areas by Federov, Wase *et al.* and Wechsler and Roizin is to our minds due to the fact that the analysis was done by these various authors from 6 to 24 hours after the injection of the drug.

Finally, does the concentration of the drug in a certain part of the brain signify a selective site of action?

It is noted from our results that there is a specific localization in the distribution of the drug in the various parts of the brain. Could this determine the pharmacological action of the drug? Comparing our data with the reported pharmacological and EEG studies in man and animals they would indicate a selective site of action.

The drug therefore has a selective site of action mainly on the reticular formation of brain stem and to a lesser extent on cerebral cortex. Although the concentration of the drug in the cortex is less than in the brain stem yet it is significant. It is noticed that it is more concentrated in the sensory part of the cortex (temporal and parieto-occipital) than in the motor part of the cortex (frontal lobe). This coincides with the observation of Bremer and Terzoulo (1953) which suggests that the drug acts on the sensory area of projection which determines the onset or cessation of sleep.

Summary

1. The uptake of phenothiazine S^{35} is demonstrated in the different parts of the brain of cats, monkey and human being by radio assay and auto-radiographic technique.

2. Comparative data are tabulated in Tables I and II showing lower uptake in the cortex than in the brain stem after 2 hours of injecting the dye.

3. The ratio of the uptake at 2 hours and 24 hours after injecting the drug in the experimental animals shows the main action of the drug to be in the brain stem.

4. Auto-radiography shows the selective section of the drug is demonstrated on the reticular formation.

5. A possible action on the sensory cortex responsible for induction of sleep is also suggested.

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References

Arrigoni-Martelli, E. and Kramer, M. (1958). *Minerva Med.* (*Torino*), **49,** 2007.
Balestrieri, A. and Fadiga, E. (1954). *Boll. Soc. Ital. biol. sper.*, **30,** 1241.
Barlow, C. F., Schoolar, J. C. and Roth, L. J. (1957). *Neurology*, **7,** 820.
Berger, Wechsler, M. and Roizin, L. (1960). *J. Ment. Sci.*, **106,** 1501.
Bray, G. A. and Funkenstein, D. H. (1958). *Dis. Nerv. Syst.*, **14,** 216.
Bremer, F. and Terzoulo, C. (1953). *Arch. Internat. de Physiol.*, **61,** 86.
Brunson, J. G., Kalina, R. E. and Eckman, P. L. (1959). *Univ. Minn. Med. Bull.*, **30,** 186.
Carreras, M. and Angeleri, F. (1957). *Int. Rec. Med.*, **170,** 67.
Idem, and De Risio, C. (1953). *Ateneo Parmense*, **24,** 734.
Cathala, H. P. and Pocidalo, J. J. (1953). *Semaine des Hôp. Paris*, **29,** 490.
Cazzullo, C. L. and Guareschi, A. (1954). *Riv. Neurol.*, **24,** 602.
Courvoisier, S., Fourner, J., Decort, R., Kelskey, M. and Koetshet, P. (1953). *Arch. Internat. de Pharmacodyn. et de Thérap.*, **92,** 305.
Dasgupta, S. R. and Werner, G. (1954). *Arch. Internat. de Pharmacodyn et de Thérap.*, **99,** 541; *Brit. J. Pharmacol.*, **9,** 389.
De Risio, C. and Manghi, E. (1954). *Boll. Soc. Ital. biol. sper.*, **30,** 1352.
Federov, N. A. (1958). *Zh. Nevropat. i Psikhiat.*, **58,** 137.
Hiebel, C., Bonvallet, M. and Dell, P. (1954). *Semaine des Hôp. Paris*, **30,** 2347.
Martin, W. R. and Eades, C. G. (1960). *Psychopharmacologia*, **1,** 303.
Salzman, N. and Brodie, B. (1956). *J. Pharmacol.*, **118,** 46.
Terzian, H. (1952). *Rassegna di Neurol. veget.*, **9,** 211.
Tislow, R. (1961). *Dis. Nerv. Syst.*, **22,** 7.
Wase, A. W., Christensen, J. and Polley, E. (1956). *Arch. Neurol. Psychiat.*, **75,** 54.
Zakrzewska, F. (1957). *Neurol. Neurochir., Psychiat. Pol.*, **7,** 707.

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