Progressively lower levels of activity are obtained in burrowing larval, prepupal and pupal preparations. Feeding larvae are more suitable than late instar larvae as sources of microsomal oxidases, since they yield preparations with higher specific activity and, additionally, the levels of larval oxidase are relatively stable during the feeding period.[2]

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REFERENCES

1. R. I. KRIEGER and C. F. WILKINSON, *Biochem. Pharmac.* **18,** 1403 (1969).
2. R. I. KRIEGER, Ph.D. Dissertation, Cornell University (1970).
3. R. I. KRIEGER and C. F. WILKINSON, *Biochem. J.* **116,** 781 (1970).
4. M. A. Q. KAHN, *J. Econ. Ent.* **62,** 723 (1969).
5. R. J. KUHR, *J. agric. Fd Chem.* **18,** 1023 (1970).
6. R. L. WILLIAMSON and M. S. SCHECHTER, *Biochem. Pharmac.* **19,** 1719 (1970).
7. J. FUKAMI and T. SHISHIDO, *Botyu-Kagaku* **28,** 63 (1963).
8. J. R. S. FINCHAM, *J. gen. Microbiol.* **11,** 236 (1954).
9. D. GILMOUR, *Biochemistry of Insects*, p. 247, Academic Press, New York (1961).
10. H. S. MASON, in *Advances in Enzymology* (Ed. F. F. NORD), Vol. 16, p. 105. Interscience, New York (1955).

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Regional distribution of persistently bound reserpine in rat brain*

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RECENT reports from this laboratory dealing with the specific binding of reserpine have focused on binding of the drug in peripheral organs.[1–3] The present communication describes the regional distribution of reserpine in rat brain after intravenous administration. Evidence is presented for the specificity of persistent reserpine binding in brain.

Female Sprague–Dawley rats, 160–180 g, received intravenously 200 μg/kg of [^{3}H]reserpine (New England Nuclear Corp., 424 mc/m-mole, labeled in the trimethoxybenzoic acid moiety). The rats were killed by chloroform asphyxiation 6, 18 or 42 hr after drug administration. Brains were removed and dissected into seven regions as described by Glowinski and Iversen,[4] the regions being cerebellum, medulla-pons, striatum, midbrain, hypothalamus, cerebral cortex, and hippocampus. The concentration of [^{3}H]reserpine was measured in each region as described previously.[1]

Concentrations of [^{3}H]reserpine in certain parts of rat brain such as striatum and midbrain were quite variable. As shown in Table 1, the lowest [^{3}H]reserpine concentrations were found in the cerebellum while the highest concentrations were found in the striatum. Levels in the striatum at 18 hr, however, did not differ significantly from those in the medulla, and levels in the cortex did not differ significantly from those in the midbrain. There was relatively little change in the observed concentrations in the various regions between 6 and 42 hr.

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TABLE 1. REGIONAL DISTRIBUTION OF [^{3}H]RESERPINE IN RAT BRAIN AT VARIOUS TIMES AFTER ADMINISTRATION*

Brain area	[^{3}H]Reserpine (ng/g ± S.E.M.) Time after administration 6 hr (3)	18 hr (5)	42 hr (3)
Cerebellum	1·52 ± 0·38	0·66 ± 0·23	0·83 ± 0·24
Cortex	3·08 ± 0·52	2·51 ± 0·24	2·84 ± 0·71
Hippocampus	2·78 ± 0·35	1·81 ± 0·08	1·87 ± 0·48
Midbrain	3·79 ± 1·34	3·23 ± 0·25	3·25 ± 1·16
Medulla	4·57 ± 0·60	4·18 ± 0·62	3·15 ± 0·55
Striatum	8·23 ± 5·83	7·02 ± 1·36	5·30 ± 2·23
Hypothalamus	1·95 ± 0·70	3·04 ± 1·24	2·40 ± 0·20

* Rats were given [^{3}H]reserpine (200 μg/kg, i.v.) and were killed 6, 18 or 42 hr later. Brain regions from two rats were pooled for each experiment. Figures in parentheses denote number of experiments.

There was no clear correlation between the relative distribution of reserpine and that of any single endogenous monoamine. In rat brain, dopamine is localized almost exclusively in the striatum,[4] but although the highest [^{3}H]reserpine concentrations were found there, other brain areas were about as high. Similarly, no clear correlation existed with the distribution of norepinephrine, which is highly localized in the hypothalamus with lower levels in the medulla-pons and very little in the other areas.[4] Serotonin is distributed more evenly than are the catecholamines in rat brain. Highest concentrations are found in the hypothalamus and medulla, and lowest in the cortex and cerebellum,[5] whereas at all times measured reserpine concentrations were about as high in cortex as in hypothalamus. As all three of the monoamines are affected by reserpine, it might be that reserpine localization in brain is dictated by the distribution of axons containing any one of the monoamines. Again however, it should be pointed out that the cortex, which contains little of any of the amines, bound appreciable concentrations of reserpine.

TABLE 2. EFFECT OF PRETREATMENT WITH TETRABENAZINE OR UNLABELED RESERPINE ON [^{3}H]RESERPINE CONCENTRATIONS AND DISTRIBUTION IN RAT BRAIN*

Brain area	[^{3}H]Reserpine (ng/g ± S.E.M.) Control (5)	Tetrabenazine pretreated (3)	Unlabeled reserpine pretreated (3)
Cerebellum	0·66 ± 0·23	0·48 ± 0·05	0·51 ± 0·05
Cortex	2·51 ± 0·24	0·97 ± 0·08	0·92 ± 0·07
Hippocampus	1·81 ± 0·08	0·88 ± 0·19	0·59 ± 0·07
Midbrain	3·23 ± 0·25	0·94 ± 0·33	0·54 ± 0·02
Medulla	4·18 ± 0·62	1·07 ± 0·19	0·82 ± 0·08
Striatum	7·02 ± 1·36	1·09 ± 0·17	1·72 ± 0·65
Hypothalamus	3·04 ± 1·24	0·92 ± 0·15	1·06 ± 0·51

* Rats were given tetrabenazine (20 mg/kg, i.p.) 30 min before, or unlabeled reserpine (0·5 mg/kg, i.m.) 6 hr before [^{3}H]reserpine (200 μg/kg, i.v.). Others were given the labeled drug only. All animals were killed 18 hr after [^{3}H]reserpine administration. Brain regions from two rats were pooled for each experiment. Figures in parentheses denote number of experiments.

The specificity of reserpine binding in the various areas was examined in two ways. One was by taking advantage of evidence that tetrabenazine shares the same receptor as reserpine. Thus it has been shown that pretreatment with tetrabenazine not only inhibits the persistent pharmacologic effects of reserpine,[6] but also lowers the concentration of persistently bound reserpine in tissues.[1,7] Accordingly, rats were given tetrabenazine (20 mg/kg, i.p.) 30 min before [^{3}H]reserpine and were

killed 18 hr after administration of the labeled drug. Analysis of the various brain areas showed that with the exception of the cerebellum, residual [^{3}H]reserpine concentrations were markedly lowered in all brain areas (Table 2), indicating that mutual reserpine-tetrabenazine sites are widespread in the brain.

The other way of establishing the specificity of reserpine binding was by taking advantage of the observation that specific reserpine binding sites are readily saturated and that pretreatment with unlabeled reserpine well in advance of the labeled drug greatly reduces the persistent binding of the latter.[1] Accordingly, rats were given unlabeled reserpine (0·5 mg/kg, i.m.) 6 hr before [^{3}H]reserpine and were killed 18 hr after administration of the labeled compound. As was the case with tetrabenazine pretreatment, concentrations of [^{3}H]reserpine were markedly lowered in all areas except the cerebellum (Table 2).

These findings suggest that specific reserpine binding sites are widespread in the brain and that they are not well correlated with the anatomical distribution of any single brain monoamine although the degree of reserpine binding in peripheral organs seems to be correlated with the degree of adrenergic innervation.[1] The present results suggest further that specific and persistent reserpine binding in cortex, which has a low content of any of the endogenous monoamines, may reveal the presence of monoaminergic systems not associated with large amine storage pools. Other evidence that there may exist such systems has been presented by Snyder and Coyle[8] who showed that the cerebral cortex, a norepinephrine-poor area, takes up norepinephrine *in vitro* almost as well as the norepinephrine-rich hypothalamus. If such hidden systems should exist, it would follow that the subcellular site of reserpine binding in brain may not necessarily be limited only to amine storage granules within monoaminergic neurones or, alternatively, that in some brain regions such granules have a low monoamine content.

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REFERENCES

1. H. S. ALPERS and P. A. SHORE, *Biochem. Pharmac.* **18,** 1363 (1969).
2. S. NORN and P. A. SHORE, *Biochem. Pharmac.* **20,** 1291 (1971).
3. S. NORN and P. A. SHORE, *Biochem. Pharmac.* **20,** 2134 (1971).
4. J. GLOWINSKI and L. L. IVERSEN, *J. Neurochem.* **13,** 655 (1966).
5. R. P. MAICKEL and F. P. MILLER, *Adv. Pharmac.* **6A,** 71 (1968).
6. G. P. QUINN, P. A. SHORE and B. B. BRODIE, *J. Pharmac. exp. Ther.* **122,** 295 (1958).
7. L. MANARA and S. GARATTINI, *Eur. J. Pharmac.* **2,** 139 (1967).
8. S. H. SNYDER and J. T. COYLE, *J. Pharmac. exp. Ther.* **165,** 78 (1969).

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Inhibition of cholesterol side-chain cleavage by azacholesterols*

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INHIBITORS of cholesterol side-chain cleavage are of interest because of their possible diagnostic and therapeutic utility in diseases associated with hyperfunctioning adrenal glands.[1] One such inhibitor, aminoglutethimide (I), is known to block the conversion of cholesterol (III) to 20α-hydroxycholesterol

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